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Diseases, Parasites, and Toxic Responses of Commercial Penaeid Shrimps of the Gulf of Mexico and South Atlantic Coasts of North America

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Noninfectious disease agents in penaeid shrimps are chemical pollutants, heavy metals, and environmental stresses. Organochlorine, organophosphate, and carbamate pesticides all have adverse effects in penaeids. Fractions of petroleum, particularly the napthalenes, are very toxic to shrimp. Little other work has been done on the effects of petroleum on penaeid shrimps. Cadmium causes black gills in shrimp by killing gill cells. Mercury is accumulated by penaeios and may interfere with their osmoregulatory abilities. Many chemotheropeutic chemicals used routinely in treatment of fish diseases are toxic to shrimp at certain determined concentrations.

Spontaneous pathoses found are a benign tumor, muscle necrosis, and gas bubble disease. "Shell disease" is discussed from points of view of possible causes. A syndrome of "broken backs" is reported in penaeid shrimps for the first time. An overview is presented for general needs in penaeid shrimp health research.

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Fungi	Pathogenesis	Noninfectious agents	
Virus	Pesticides	Spontaneous pathoses	
Protozoa	Metals		
Helminthes			
Nematodes			

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DISEASES, PARASITES, AND TOXIC RESPONSES OF COMMERCIAL PENAEID SHRIMPS OF THE GULF OF MEXICO AND SOUTH ATLANTIC COASTS OF NORTH AMERICA[1]

JOHN A. COUCH[2]

ABSTRACT

A reference work and review of both infectious and noninfectious diseases of commercial penaeid shrimps of the Gulf and South Atlantic region of the United States is presented. Disease is second only to predation and periodic physical catastrophes in limiting numbers of penaeid shrimps in nature and second only to nutritional and reproductive requirements in limiting aquacultural successes with penaeid shrimps.

Infectious agents causing disease in penaeid shrimps are a virus, bacteria, fungi, protozoa, helminthes, and nematodes. A well-described *Baculovirus* infects larval and adult shrimp and is associated with mortality, particularly in larval shrimp. Bacteria of the genera *Vibrio, Beneckea,* and *Leucothrix* are associated with disease in penaeid shrimps, but bacterial roles in mortality are unclear. The same is largely true for fungi with members of the genera *Lagenidium* and *Fusarium* causing pathogenesis in cultured shrimp. *Lagenidium* causes severe destruction of larval shrimp tissues. Of the many protozoan groups represented in and on penaeid shrimps as tissue parasites and commensals, the Microsporida of the genera *Nosema, Thelohania,* and *Pleistophora* are the most destructive. The ciliate protozoa *Zoothamnium* sp., *Lagenophrys* sp., and *Parauronema* sp. may cause dysfunction in shrimp. An undescribed apostome ciliate is associated with black gill disease. A suctorian, *Ephelota* sp., is an ectocommensal of larval shrimp, attaching to the cuticle. The six species of gregarines reported cause little or no pathogenesis, and a single reported flagellate species role in shrimp health is uncertain.

Flatworms found in penaeid shrimps are metacercariae of a species of *Microphallus* in muscles and viscera, metacercariae of *Opecoeloides fimbriatus* in viscera, plerocercoid larvae of *Prochristianella hispida* in the hepatopancreas and hemocoel, and four other cestode developmental stages. Nematodes found are *Thynnascaris* sp., *Spirocamallanus pereirai, Leptolaimus* sp., and *Croconema* sp.

Noninfectious disease agents in penaeid shrimps are chemical pollutants, heavy metals, and environmental stresses. Organochlorine, organophosphate, and carbamate pesticides all have adverse effects in penaeids. Fractions of petroleum, particularly the naphthalenes, are very toxic to shrimp. Little other work has been done on the effects of petroleum on penaeid shrimps. Cadmium causes black gills in shrimp by killing gill cells. Mercury is accumulated by penaeids and may interfere with their osmoregulatory abilities. Many chemotheropeutic chemicals used routinely in treatment of fish diseases are toxic to shrimp at certain determined concentrations.

Spontaneous pathoses found are a benign tumor, muscle necrosis, and gas bubble disease. "Shell disease" is discussed from points of view of possible causes. A syndrome of "broken backs" is reported in penaeid shrimps for the first time. An overview is presented for general needs in penaeid shrimp health research.

Recent attempts to culture penaeid shrimps in large quantities have stimulated renewed interest in the pathobiology of crustacean species. Pathogens and disease, in general, have been indicted as causes for many failures in maintaining various life-cycle stages of Crustacea. Therefore, considerable amounts of new information and data on known and recently discovered diseases of penaeid shrimps have been published or reported in the last decade. This recent information, along with an older but equally valuable series of publications, presents a substantial body of knowledge which describes and defines problems of disease encountered in the biology, management, and massive culture of penaeid shrimps.

Major contributions to the study of shrimp diseases in North America have been made by sev-

[1]Contribution No. 283 from the Gulf Breeze Environmental Research Laboratory.

[2]U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, FL 32561.

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eral individuals. Sprague (1954, 1970, footnote 3), Kruse (1959, 1966), Hutton et al. (1959), Iversen and Manning (1959), Hutton (1964), and Iversen and Van Meter (1964) were early explorers in penaeid shrimp infectious diseases. More recently the works of Overstreet (1973), Lightner (1974, 1975), Lightner and Fontaine (1973), Johnson (1974), Feigenbaum (1973, 1975), Couch (1974a, b, 1976) and Sindermann[4] have contributed to the general fund of data. Overstreet's 1973 paper is particularly valuable because it gives prevalence data for many of the parasites of penaeid shrimps of the northern Gulf. Many other authors of single, significant works on penaeid diseases will be cited in specific sections later in this paper.

The scientific reports and reviews mentioned above, along with much unpublished experience, present a consensus which impresses me with the high significance of disease to the overall ecology and biology of penaeid shrimps. In its broadest sense, disease is probably second only to predation and periodic physical catastrophes (e.g., freshets, temperature fluctuations) as a continuous environmental factor limiting numbers of penaeid shrimps in nature. In attempts at massive culture of penaeid shrimps, infectious disease may rank below only reproductive and nutritional requirements as a limiting factor. Toxicants, in the form of pollutants, are threats to the well being of estuarine species, particularly in certain chronically polluted regions. Toxic responses in penaeid shrimps have been studied experimentally recently, and, therefore, some data are available on this subject.

This paper is concerned with the present status of diseases, parasites, and toxic responses of four commercial species of penaeid shrimps from the Gulf and South Atlantic region of North America. These are the pink shrimp, *Penaeus duorarum*; the brown shrimp, *P. aztecus*; and the white shrimp, *P. setiferus*. Occasional reference will be made to parasites of *P. braziliensis* which occupies a marginal portion of the U.S. range of the three other species. The subjects will be treated in the following order: Infectious diseases and parasites; noninfectious diseases and toxic responses; and overview and future research.

[3]Sprague, V. 1950. Notes on three microsporidian parasites of Decapod crustacea from Louisiana waters. Occas. Pap. Mar. Lab., La. State Univ. 5:1-8.

[4]Sindermann, C. J. 1974. Diagnosis and control of mariculture diseases in the United States. Tech. Ser. Rep. No. 2, Natl. Mar. Fish. Serv., NOAA, Highlands, N.J., 306 p.

INFECTIOUS DISEASES AND PARASITES

Viruses

To date, only a single virus disease has been described for shrimps. Couch (1974a, b) and Couch et al. (1975) have described a rod-shaped virus (Figures 1-3) which has many characteristics of the baculoviruses (nuclear polyhedrosis viruses) previously described only from insects or mites. The virus has been named *Baculovirus penaei* (Couch 1974b).

This virus commonly has been found to infect the hepatopancreas of juvenile and adult stages of pink and brown shrimp in nature. Laboratory-reared larval brown shrimp (protozoea and mysis stages) have been found with virus-infected midgut and hepatopancreas.

Infected hepatopancreatic cells in pink shrimp display striking cytopathological changes when compared with normal, noninfected cells. Nuclear hypertrophy (Figure 3), chromatin diminution (Figure 3), nucleolar degeneration (Figure 3), and polyhedral inclusion body (PIB, Figure 2) production are characteristic of patent virus infections observable with bright field or phase contrast microscopy.

Electron microscopy (EM) reveals the rod-shaped virions (269 nm × 50 nm) in infected, hypertrophied nuclei prior to, during, and after the PIB is formed. Various stages of the virus replicative cycle are observable with EM of thin sections of moderately to heavily infected hepatopancreas. The ultimate cytopathological effect of the virus is destruction of the host cell through rupture or lysis. This is accomplished usually by the growth of the PIB to a size too large for the host cell to accommodate (Figure 4), concomitant with virus-induced nuclear hypertrophy and probable stressing of nuclear membranes.

The PIB's produced during infections are patently diagnostic for the baculovirus of penaeid shrimp (Figures 4, 5). To find a single characteristic PIB in tissue squashes of shrimp hepatopancreas or midgut is to diagnose infection. Quantitation of patent infections (PIB's present) can be made on a relative basis by hemocytometer counts of PIB's in aliquots of fresh tissue. Degree of latent infections, however, may be estimated only with great difficulty through laborious EM examinations. Over 2,000 PIB's/mm^3 of hepatopancreatic tissue are considered a heavy infection as determined by hemocytometer counts. Heavy patent

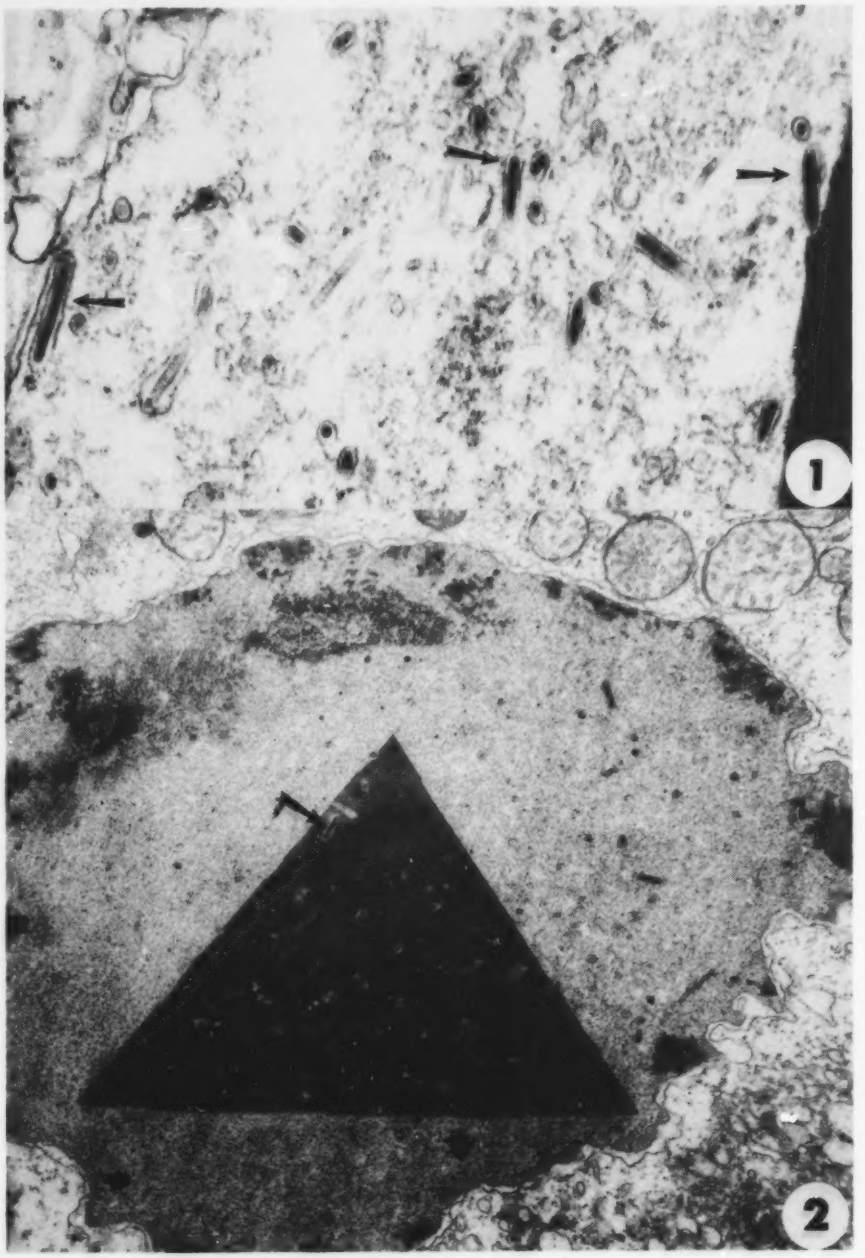


FIGURE 1.—*Baculovirus* virions in nucleus of hepatopancreatic cell of pink shrimp; note rod form (arrows) and outer envelope surrounding nucleocapsid (electron micrograph). ×70,000.

FIGURE 2.—Polyhedral inclusion body (PIB) in virus-infected nucleus; note characteristic triangular form, and rod-shaped virions in PIB (arrows); also note heterochromatin diminution and granular nucleoplasm. ×22,260.

FIGURE 3.—Two hepatopancreatic cells with *Baculovirus*-infected nuclei; note nuclear membrane proliferation (arrow) and nuclear hypertrophy. ×14,400.

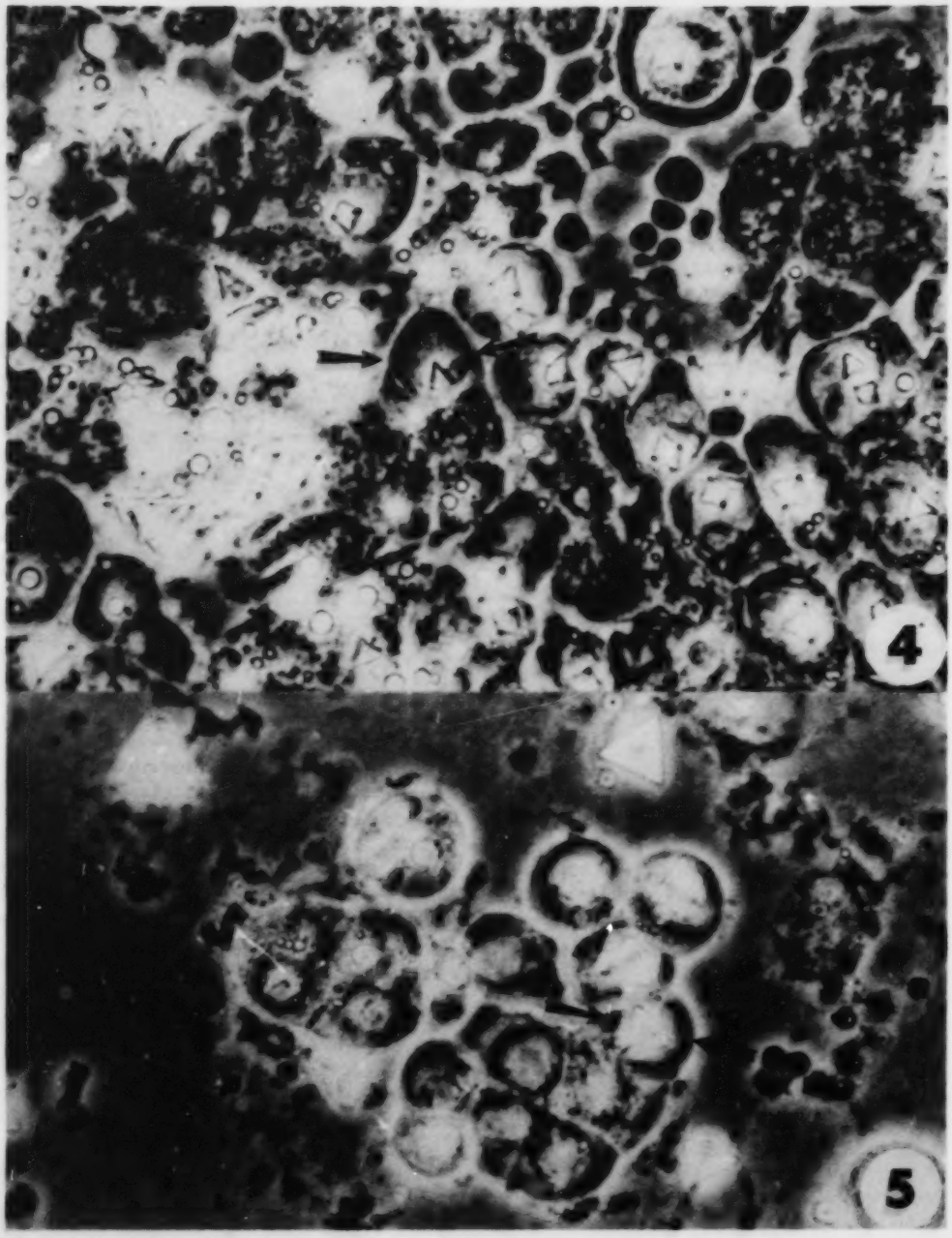


FIGURE 4.—Phase contrast micrograph of fresh squash preparation of heavily, patent, virus-infected hepatopancreas from pink shrimp, note hypertrophied nuclei (arrows) and characteristic refringent PIB's. ×1,000.

FIGURE 5.—Phase contrast micrograph of fresh squash showing PIB's (arrows) of varying sizes, some free of nuclei following nuclear rupture. ×1,000.

infections are obvious in fresh squash preparations because PIB's fill every microscopic field.

Prevalence of virus in feral pink shrimp from several locations on the northern gulf coast of Florida has varied among samples collected. There appears to be no seasonal intensification of prevalence that is statistically significant; however, fall samples have been best for recovering heavy infections. To date, of 4,676 shrimp examined, 808 have been patently infected. In the laboratory, virus prevalence and intensity have increased repetitively in 20- to 30-day periods in different lots or samples of feral shrimp held under crowded, sublethally stressful conditions (Couch 1974b). This increase in prevalence associated with crowding provides indirect evidence for the infectious nature of the shrimp baculovirus. There is also increasing evidence, from our research, that exposures to low levels of certain chemicals, such as polychlorinated biphenyl (PCB), enhance spread of virus through captive populations (Couch and Courtney 1977). We have induced a 50% increase in prevalence in captive shrimp by exposing shrimp to sublethal levels of PCB's (Aroclor 1254).[5] Transmission in nature probably is achieved via cannibalism of infected shrimp by noninfected shrimp. Laboratory transmission has been minimally successful when hatchery-reared or nonpatently infected juvenile or adult shrimp were fed heavily infected hepatopancreas. Only about 20% of fed shrimp show patent infections 20 to 30 days after initial feeding. Degree of infection in adult shrimp is not useful in predicting mortality of shrimp.

Recently the shrimp baculovirus was associated with massive mortality of larval and postlarval brown shrimp in a commercial aquaculture attempt. Brown shrimp, hatched and reared to protozoal and mysid stages in laboratory tanks, suffered a mass mortality in a 48-h period (95% of several million larvae). Water quality was not found to be at fault and there were no toxicants known to be in the water. Upon careful histological examination of a sample of surviving and dead larvae, I discovered that 19.4% (n = 139) had patent virus infections, mostly heavy, in midgut and hepatopancreatic cells (Table 1). Subsequent electron microscopical study confirmed that 60 to 90% of hepatopancreatic cell profiles in larvae had infections, many with prepatent stages of the virus. Present in higher prevalences in these dying shrimp were a flagellate protozoon and a ciliate protozoon. The relative roles of the three pathogens in the shrimp mortality will be discussed in later sections of this paper (Tables 1, 2).

TABLE 1.—Relative prevalence of pathogens in 139 larval (late protozoeal and mysid stages) brown shrimp, *Penaeus aztecus*,[1] in April 1974.

Condition	Number of larvae affected	Percent of total examined
Not infected	41	29.5
Flagellate	89	64.0
Ciliate	40	28.8
Virus	27	19.4

[1]Whole mount slides with Protargol stain (Bodian-activated protein silver).

TABLE 2.—Prevalence and concurrent infections of pathogens in 139 larval brown shrimp examined in April 1974.
[Concurrent vs. single infections.]

Types of pathogens	Number of larvae affected	Percent of total examined
None	41	29.5
Flagellate only	38	27.3
Ciliate only	1	0.7
Virus only	8	5.8
Flagellate and ciliate	32	23.0
Flagellate and virus	12	8.6
Ciliate and virus	0	0.0
Flagellate, virus, and ciliate	7	5.0

Bacteria

The role of bacteria in diseases of penaeid shrimps is presently being investigated seriously for the first time. A few scattered reports deal with bacteria as pathogens, contaminants, or ectocommensals in shrimps.

Cook and Lofton (1973) reported isolation of three genera of bacteria, *Beneckea*, *Vibrio*, and *Pseudomonas*, from penaeid shrimp suffering from "shell disease," also known as black spot disease. This disease (Figure 6) is characterized by brown to black spots on the external carapace or cuticle of shrimp and has been observed in brown, pink, and white shrimps. In advanced cases of the disease, considerable erosion and destruction of the cuticle occurs. This disease has been reported from many other decapod Crustacea (Rosen 1970). Chitinoclastic bacteria such as *Beneckea* sp. have been thought to be the causative agents of black spot disease, although attempts to experimentally produce the disease in shrimp by innoculating *Beneckea* have had uncertain results (see section on "shell disease" under Noninfectious Diseases). Mechanical injury to shrimp that results in breakage in the normal cuticle probably plays an

[5]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA, or USEPA.

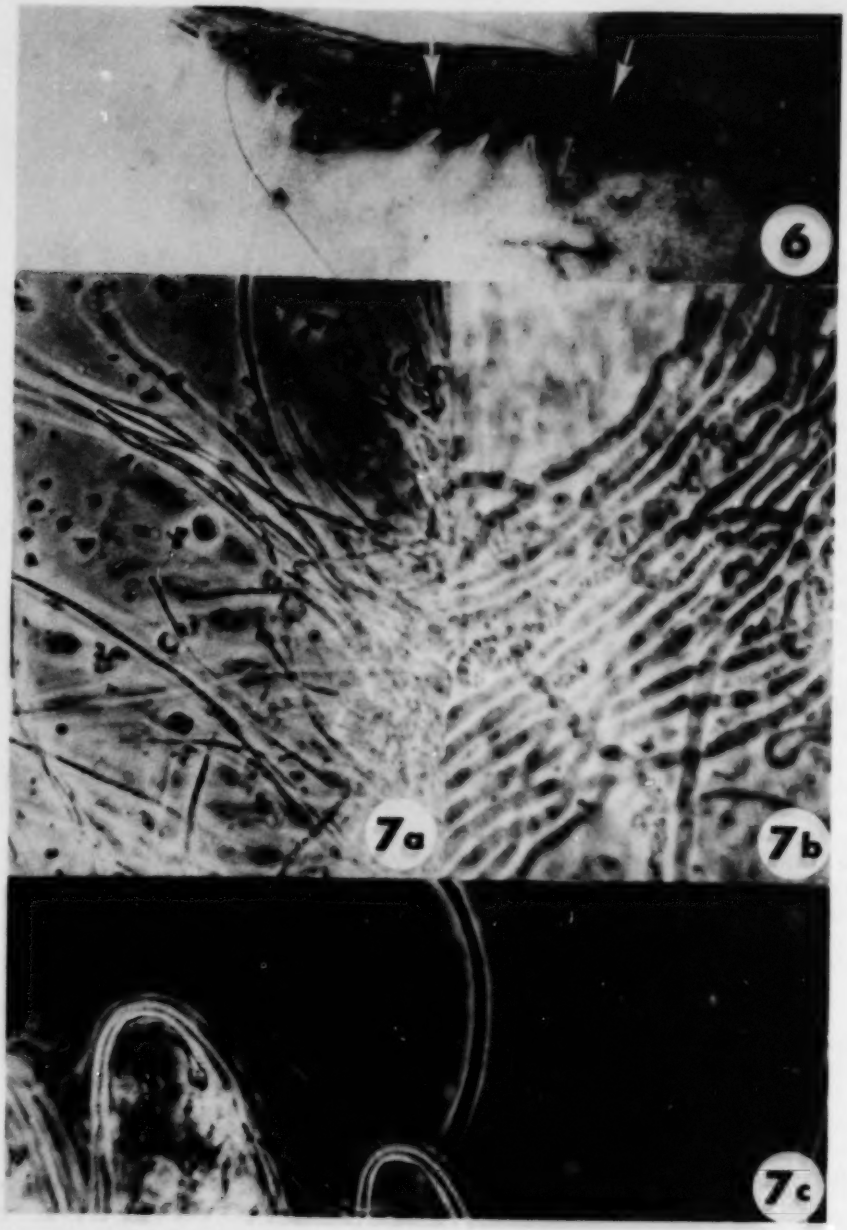


FIGURE 6.—"Shell disease" in pink shrimp; note black spots of varying sizes (arrows); none of these have penetrated cuticle of shrimp at this stage.

FIGURE 7.—a. Filaments of *Leucothrix mucor* (bacteria) in heavy infestation on gills of pink shrimp. ×400. b. Wet mount preparation of *L. mucor* from heavy gill infestation; note granules in some filaments. ×900. c. Single filaments of *L. mucor* showing attachment to end of gill filament; note few bacterial filaments in light infestation shown here. ×900.

initiating role in the genesis of black spot disease (Cook and Lofton 1973).

The effects of black spot disease on individual shrimp is apparently a breakdown of cuticular protection, thus permitting loss of hemolymph and invasion by internally destructive pathogens. Black spot disease in penaeids is fairly common, at least in early manifestation. However, the disease probably plays a minor role in mortalities of feral shrimp because shrimp probably tolerate the initial lesions well.

Vanderzant et al. (1970) isolated *Vibrio parahemolyticus* from white shrimp from the Gulf of Mexico. This bacterium is one etiological agent for human gastroenteritis in Japan and possibly in the United States (Krantz et al. 1969). The pathogenicity of *V. parahemolyticus* for Crustacea, including shrimp, has not been conclusively established. One should remember that natural seawaters, particularly from inshore regions, may be considered "gram negative bacterial soups." Therefore, the presence of *Vibrio* sp. and other gram negative rods on marine organisms living in the "soup" should be expected. The role that *Vibrio* plays in the health of shrimps is uncertain. Ulitizur (1974) has pointed out that certain strains of *Vibrio parahemolyticus* isolated from seawater have very short generation times (12-14 min) at higher temperatures (39°C). In subtropical areas where temperatures might soar in hot seasons, particularly in ponds, the role of *Vibrio* sp. as pathogens of shrimp might be enhanced. Lightner (1975) discussed at length the suspect role of *Vibrio* spp. in penaeid shrimp health.

Ectocommensal bacteria may play a significant role in the well being of penaeids, particularly those held in crowded volumes of water where rich organic substrate and optimum temperatures prevail. Pertinent among this group is the filamentous bacterium *Leucothrix mucor* (Oersted), a widespread epiphyte of marine animals and plants (Johnson et al. 1971). *Leucothrix* has been found in high numbers attached to the gill filaments of brown, white, and pink shrimp (Figure 7 a, b). The filaments are nonbranching, attached singly to the cuticle of the gills (Figure 7c), have a modal diameter of 2 μm, and consist of septate chains of almost square-shaped bacteria. Each bacterium has several mesosomes along its cytoplasmic membrane (Figure 8).

A study was conducted with EM to determine the mode of attachment of *Leucothrix* to shrimp gill cuticle. Figure 9a, b shows cross sections of a basal portion of a filament at its point of adhesion to gill cuticle. The bacterium does not possess a differentiated holdfast. There is no penetration of the epicuticle, and apparently the filament is secured to the gill epicuticle by an electron-opaque mucouslike substance. I presume that this substance is secreted by the bacterium.

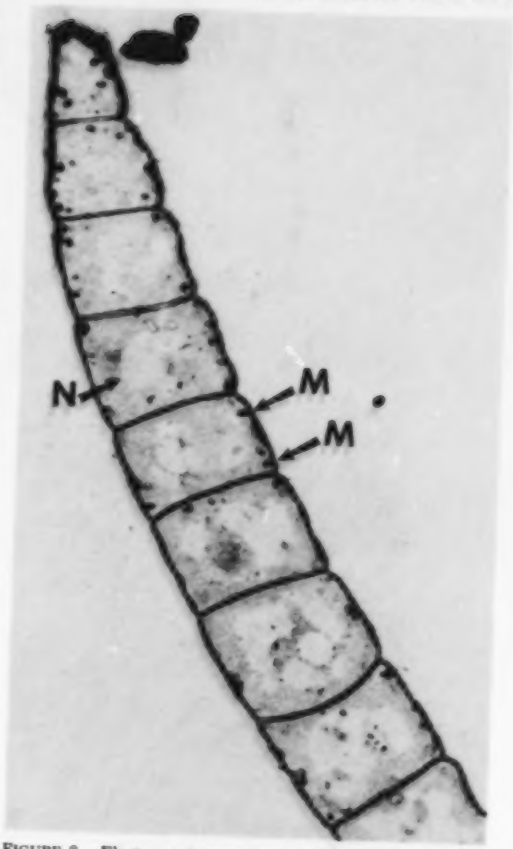
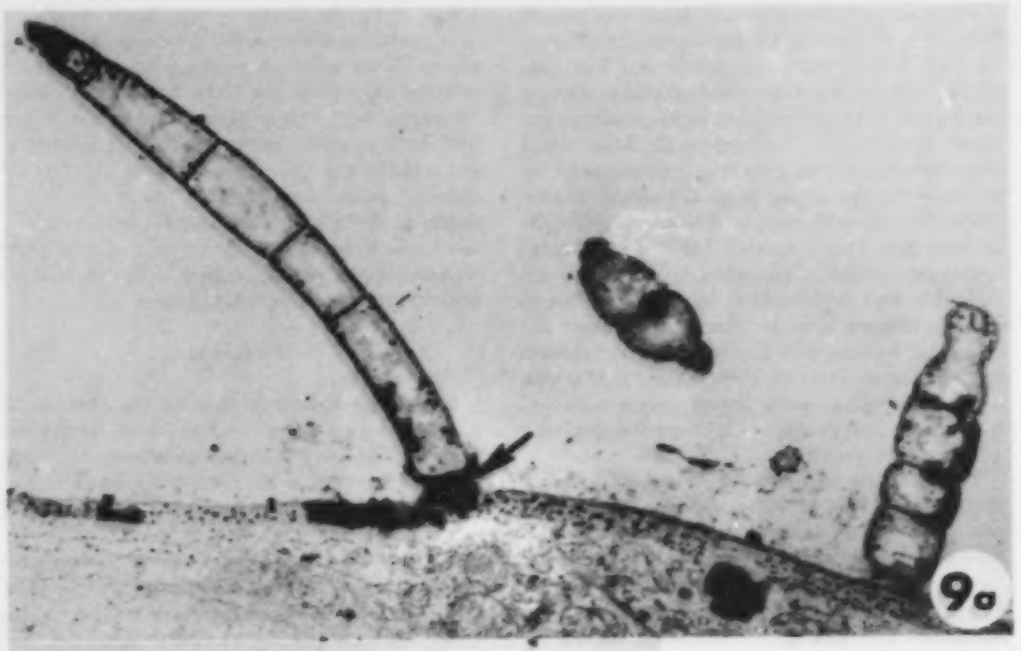


FIGURE 8.—Electron micrograph of single filament of *Leucothrix mucor* showing nearly square cell profiles; note nucleoids (N) and mesosomes (M) (arrows) of bacterial cells plus septa separating each cell in filament ×25,900.

Leucothrix grows best on penaeid shrimps when the shrimps are crowded and when there is a rich organic seawater medium. Salinities of 20-35‰ and temperatures of 20°-25°C have been adequate for overgrowths of *Leucothrix* on gills of shrimp. Terminal gonidia were not searched for or observed in the fresh natural infestations on shrimp that I have studied with phase contrast, bright field, and electron microscopy.

The major adverse effect of *Leucothrix* infestations on shrimp is probably interference with gas diffusion across gill cuticle, particularly in massive infestations (Figure 7a, b). In experiments at my laboratory, I found that pink shrimp when exposed to various levels of an ethylene glycol-containing waste in bioassay systems had heavy growths of *L. mucor* on their gills, whereas nonexposed, control shrimp had little or no growth on their gills. Mortality of the exposed shrimp was proportionate to the extent of growth of *Leucothrix* on their gills. Indications from EM studies are that the mucoid substance with which *L. mucor* attached to gills may cover gills (Figure 9a, b) in



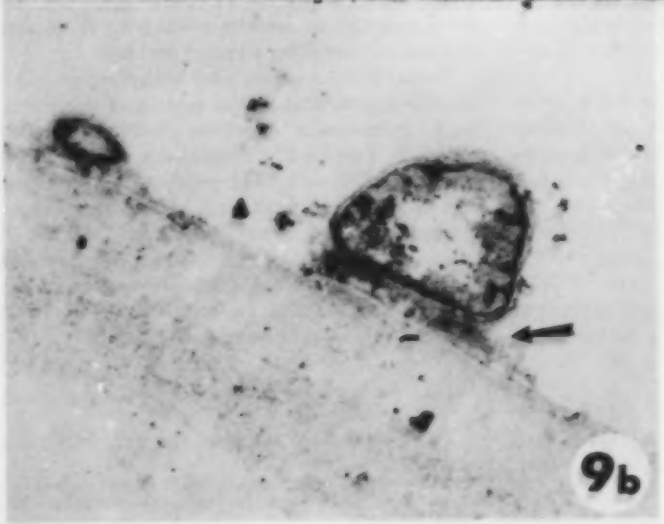


FIGURE 9.—a. Relationship of *Leucothrix mucor* filaments to gill cuticle of pink shrimp; note electron-opaque mucoid substance (arrow) at point of attachment and adjacent to base of bacterium; no penetration of cuticle occurs, demonstrating that the bacterium is not invasive. ×14,400. b. Higher magnification of *L. mucor* at point of attachment to gill; note distribution of electron-dense mucoid substance probably secreted by bacterium (arrow); shrimp cuticle is intact. ×28,500.

heavy infestations. Massive amounts of this substance overlying gill cuticle could block normal gas diffusion across gill surfaces.

Fungi

Our knowledge of fungal diseases of penaeid shrimps is in a state similar to that of our knowledge of bacterial diseases. The only clear-cut case of a fungal pathogen affecting large numbers of penaeid shrimps in the United States was reported by Cook (1971) and by Lightner and Fontaine (1973). These authors described infections of white shrimp larvae by a phycomycete, *Lagenidium* sp., an estuarine fungus. The fungus infects the second protozoal stage of white shrimp, and disappears by the time the first mysis stage is reached. Figure 10a shows a heavily infected protozoea. According to Lightner and Fontaine (1973), the major pathogenic effect is almost complete tissue destruction and replacement by invasive fungal mycelia (Figure 10a). Hyphae of the fungus are branched, septate, with thin walls, and range from 8.0 to 11 μm in diameter. Under bright field microscopy the hyphae were yellow-green and contained round oil droplets (Lightner and Fontaine 1973).

The lifecycle of *Lagenidium* sp. in penaeid shrimps involves a sporulation phase. This begins when a hyphal extension penetrates the cuticle of the shrimp from within (Figure 10b). Following formation of a vesicle in the apical region of the extension, planonts (flagellated zoospores) are formed in the vesicle. The whole extension becomes a discharge tube, releasing motile planonts (8.7-12 μm) which presumably infect other shrimp.

Lightner and Fontaine (1973) were able to infect larval brown shrimp (protozoea I) with planonts and hyphae on a large scale (2,000 larvae). Resulting mortality in the experimentally infected shrimp was 20%. Approximately 60 h were required for infections to become patent. The role of this fungus in natural shrimp populations is not known. In aquaculture the fungus could be a definite limiting factor in the survival of shrimp larvae. Brown shrimp larvae in commercial hatcheries have been found to die of this disease (Cook 1971).

The only other report of natural fungal infection in penaeid shrimps in the United States was that of Johnson.[6] He briefly described a *Fusarium* species which infected the gills and antennal scales of *Penaeus duorarum*. Less than 5% of shrimp studied were infected and the spread of the fungal mycelium in the body of affected shrimp was slow.

Solangi and Lightner (1976) have described the cellular inflammatory response of *Penaeus aztecus* and *P. setiferus* to experimental infections of *Fusarium* sp. According to these authors, both species of shrimps showed "complete resistance to infection by the fungal spores when normal or wounded shrimp were held in seawater containing the spores or when spores were injected directly into the shrimp in low concentrations." Cellular "melanization" and encapsulation of the micro- and macroconidia occurred in gill tissues of penaeid shrimp. Only massive doses of 3.2×10^6 spores injected into brown shrimp resulted in death of shrimp; this lethality was a result of mechanical blockage, by spores, of the blood sinuses of the shrimp's gills. Gills of affected shrimp sometimes were blackened.

Protozoa

More than any other phylum, the Protozoa as pathogens and parasites have had significant, known effects on shellfish populations. Representatives of every class of Protozoa are found as symbionts, commensals, parasites, or pathogens in penaeid shrimps. Certain groups such as the Microsporida have a long history as pathogens of not only penaeid shrimps, but arthropods in general. Only recently, however, species of such groups as the Ciliophora and the Sarcomastigophora have been indicted as serious pathogens of decapod Crustacea, including penaeid shrimps.

Herein Protozoa associated with shrimps will be classified according to the scheme of the Honigberg Committee in "A Revised Classification of the Phylum Protozoa" (Honigberg et al. 1964). Sprague and Couch (1971) published an annotated list of protozoan parasites, hyperparasites, and commensals of decapod Crustacea. This list includes most of the known species of Protozoa associated with penaeid shrimps. However, since its publication, several undescribed species have been found and will be included herein.

Subphylum Sporozoa Leuckart 1879

This subphylum includes the gregarines and

[6] Johnson, S. K. 1974. *Fusarium* sp. in laboratory-held pink shrimp. Texas A&M Univ., Fish Disease Diagnostic Lab. Note FDDL-51, 1 p.

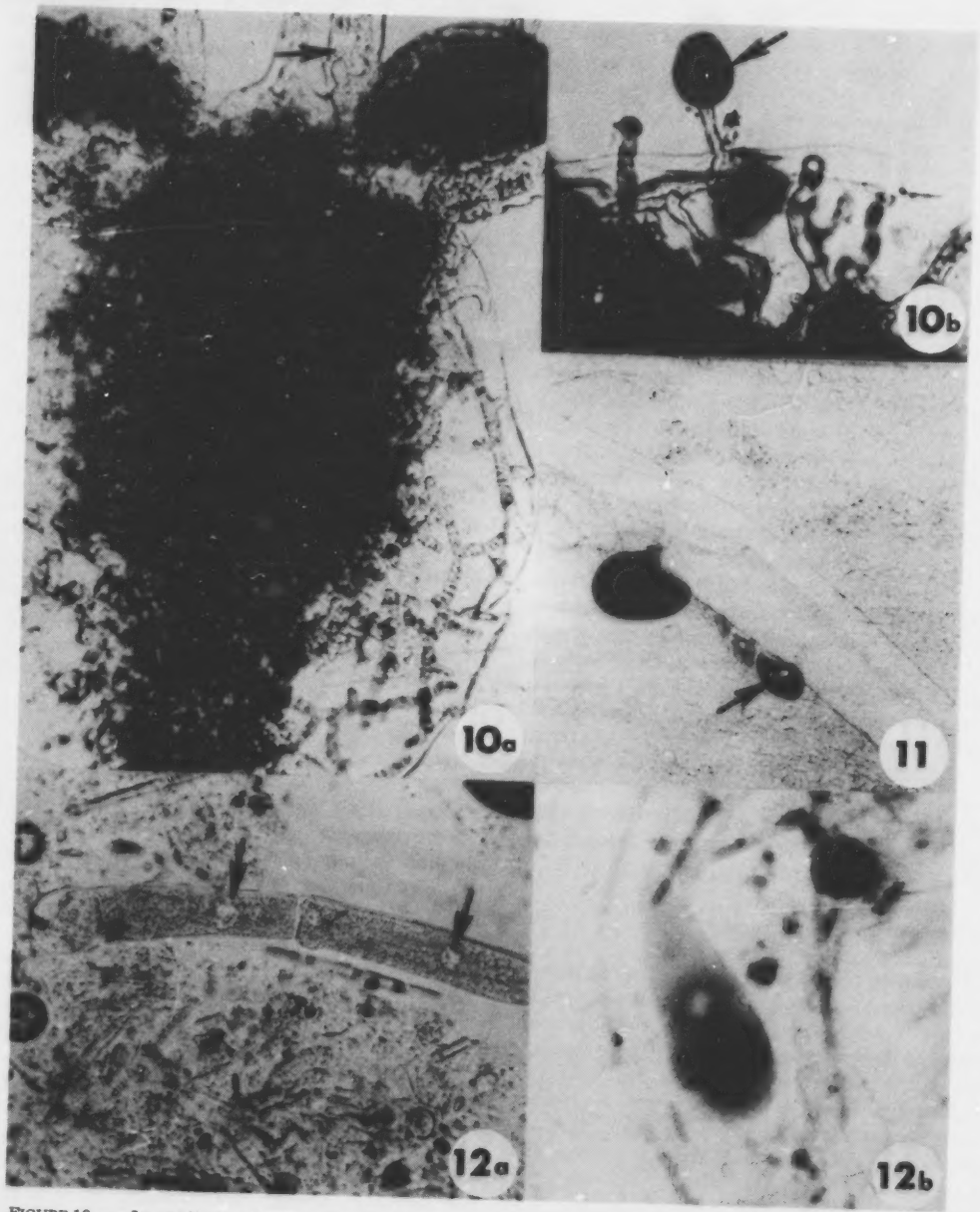


FIGURE 10.—a. *Lagenidium* sp. hyphae throughout body of larval penaeid shrimp; note fungus has invaded antenna near eye (arrow). ×300. b. *Lagenidium* sp. sporulation stage; note sporulation vesicle, filled with planonts, on end of hyphal extension (arrow) that has penetrated larval shrimp cuticle. ×400.

FIGURE 11.—*Cephalolobus penaeus*, gregarine trophonts attached to lappet of gastric mill from pink shrimp; note nucleus (arrow) and mode of attachment. ×150.

FIGURE 12.—a. *Nematopsis* sp. trophonts in syzygy, from midgut of pink shrimp; arrows point to nuclei of trophonts. ×900. b. Single, young trophont of *Nematopsis* sp. from gut of pink shrimp; note protomerite and septum separating it from rest of primite. ×1,000.

coccidians. The only group considered here are the gregarines of penaeids. Gregarines, in general, are not highly pathogenic to their hosts. Therefore, information presented here is brief and the reader is referred to referenced works for details.

Class Telosporea Schaudinn 1900
Subclass Gregarinia Defour 1828
Order Eugregarinida Leger 1900
Family Cephaloidophoridae Kamm 1922
Cephalolobus penaeus Kruse 1959

This species attaches to chitinous walls and terminal lappets of the stomach filter in *Penaeus aztecus* and *P. duorarum* (Figure 11). Usually the attached stage is a trophozoite consisting of a primite with an anterior protomerite division that is modified into a holdfast organ. The single nucleus is in the center of the primite (Figure 11). The primite, including the protomerite, is from 100 to 200 μm long. Often attached to the primite posteriorly will be 1 or 2 satellites (young trophozoites). Spores, sporozites, and cysts have not been observed. Overstreet (1973) reported this species in *P. setiferus* from Louisiana, extending its range from Florida as previously reported. I have observed this species in pink shrimp occasionally from Pensacola, Fla. This gregarine apparently has no harmful effect on the shrimp host. It may be possible that large numbers attached to the filter apparatus of the host could interfere with filtration of particles bound for the hepatopancreatic ducts or passing through the stomach.

Cephalolobus sp. Feigenbaum 1975

This form, reported from *Penaeus brasiliensis*, utilizes the stomach filter as position of attachment within host. Trophozoites consist of protomerite and deutomerite separated by a septum. As in *C. penaeus*, the anterior end is modified into a holdfast organelle. This species has been reported in shrimp only from Biscayne Bay, Fla., and differs from *C. penaeus* in that the trophozoites occur solitarily and are smaller (43-100 μm long) than those of *C. penaeus*.

Family Porosporidae Labbe 1899
Nematopsis penaeus Sprague 1954

This species has been reported from brown, pink, and white shrimps. It is found in the intestinal tract. Figure 12a, b show specimens of *Nematopsis* from the gut of a pink shrimp. These may be *N. penaeus* or *N. duorari* (see below). Works by Sprague (1954, see footnote 3), Sprague and Orr (1955), Kruse (1959, 1966), Hutton et al. (1959), and Hutton (1964) give information on hosts including the intermediate molluscan hosts, for *N. penaeus*. Overstreet (1973) discussed the prevalence and morphology of *N. penaeus* and pointed out that syzygy is multiple with up to seven trophozoites in line attached to one another reaching a length of over 0.5 mm. Characters for distinguishing *N. penaeus* and *N. duorari* are size of gymnospore and number of different molluscan intermediate hosts. No pathogenesis is associated with this form.

Nematopsis duorari Kruse 1966

This gregarine is restricted to the gut of pink shrimp. Kruse (1966) attempted to transmit it to brown and white shrimp, but could not. Figure 12a shows an immature association of a trophozoite of *Nematopsis* sp. in syzygy. Since two of the known *Nematopsis* species of penaeids appear identical in their trophozoite stages, no attempt will be made here to identify the specimens in Figure 12 to species.

Nematopsis sp. Kruse 1966

Kruse (1966) described, but did not name, this species from concurrent infections with *N. duorari* in pink shrimp in Florida. This form had smaller gymnospores than did *N. duorari*.

Nematopsis brasiliensis Feigenbaum 1975

This is a recently described species of *Nematopsis* in a penaeid shrimp. Found in the intestine of *Penaeus brasiliensis*, this species consists of both individual trophozoites and syzygies of biassociations (two trophs). It has been described from Biscayne Bay only. Hutton (1964) reported *N. penaeus* from *P. brasiliensis*. However, Feigenbaum (1973) believes that the species Hutton reported as *N. penaeus* may have been *N. brasiliensis*.

Subphylum Cnidospora Doflein 1901
Class Microsporea Corliss and Levine 1963
Order Microsporida Balbiani 1882

Microsporida are highly pathogenic to shrimps

and are probably one of the most destructive groups of pathogens to penaeid hosts. Rarely, however, have epizootics been recorded in which large numbers of penaeids have been lost to microsporidan infections. Infection prevalences in samples of penaeids from nature and aquaculture rarely exceed 10%. Due to their highly pathogenic nature, however, emphasis is placed on the importance of these protozoa to the health of penaeids. Table 3 summarizes salient characteristics of species of Microsporida discussed below. Kelley (1975) described histopathological changes in pink shrimp infected with Microsporida.

Family Nosematidae Labbe 1899
Nosema nelsoni Sprague 1950

This species is widespread, found in *Penaeus duorarum, P. aztecus,* and *P. setiferus* along the South Atlantic and Gulf coasts of the United States. The spores are found singly (one spore per sporont) in masses in infected tail muscle (Figure 13). As with certain other Microsporida, *N. nelsoni* causes white discoloration of muscle or viscera giving infected shrimp a cotton or paper-white color (Figure 14). Fishermen call these shrimp "milk" or "cotton" shrimp. The spores of *N. nelsoni* are 1.7 to 2.5 μm long by 1.0 to 1.5 μm wide. Their polar filaments are 20 to 25 μm long. This parasite kills shrimp, and massive single infections with whole musculatures affected are found (Figure 15a, b).

Thelohania penaei Sprague 1950

Members of this genus have eight spores in each sporocyst (Figure 16a, b). Found originally in the reproductive organs of *Penaeus setiferus* in Louisiana, this species has been reported from Mississippi, Texas, and Georgia. It infects muscle, gonads, and is seen grossly along the middorsal region of the abdomen and in appendages as white spots or clusters (Figures 17, 18). Spores are pyriform and occur in two size classes (2.0 to 5.0 μm long and 5.0 to 8.2 μm long). The polar filament is unusual in that it has a thin distal half and a thick proximal half. Sprague (1970) reported that this is probably the microsporidan that Viosca (1943) observed in the reproductive organs of about 90% of *P. setiferus* along the Louisiana coast in 1919. This epizootic is one of the few reported in which penaeids have suffered en masse from a microsporidan. Viosca reported that the reproductive organs of the white shrimp were destroyed by the parasite.

Iversen and Kelly (1976) reported the first successful experimental transmission of a microsporidan (*T. penaei*) in shrimp. Postlarval pink shrimp fed *T. penaei* spores, conditioned by passing through seatrout, showed tissue infections.

Overstreet (1973) reported that pink and brown shrimps reared together in ponds showed only gill infections of *T. penaei*.

Thelohania duorara Iversen and Manning 1959

This organism was first reported from *Penaeus duorarum* from the Dry Tortugas. A similar species has been reported from brown and white shrimps (Kruse 1959) in Florida. Overstreet (1973) reported that this species occurs in pink shrimp in the Mississippi Sound, and Iversen and Van Meter (1964) found it in *P. brasiliensis* in south Florida. Spores are 5.4 μm × 3.6 μm. This microsporidan parasitizes the muscle of shrimp causing white or "cotton" shrimp. The extent of impact it has on wild populations of penaeids is not understood. According to Sprague and Couch

TABLE 3.—Characteristics of Microsporida in penaeid shrimps.

Species	Spores/sporont (averages)	Spore size (μm)	Tissues	Host(s)	Locales
Nosema nelsoni Sprague 1950	1	2.0 × 1.2	Muscle	*P. aztecus* *P. duorarum* *P. setiferus*	Gulf coast Georgia coast
Thelohania penaei Sprague 1950	8	2.0 × 5.0 5.0 × 8.2	Gonads Muscle	*P. setiferus*	Gulf coast Georgia coast
Thelohania duorara Iversen and Manning 1959	8	5.4 × 3.6	Muscle	*P. aztecus* *P. duorarum* *P. setiferus*	Gulf coast Florida east coast
Pleistophora sp. Baxter et al. 1970 Constransitch 1970 Kruse (in Sprague 1970) Iversen and Kelly 1976	16 to 40+	2.6 × 2.1	Muscle Heart Gills Hepatopancreas	*P. aztecus* *P. setiferus* *P. duorarum*	Gulf coast Southeast Florida

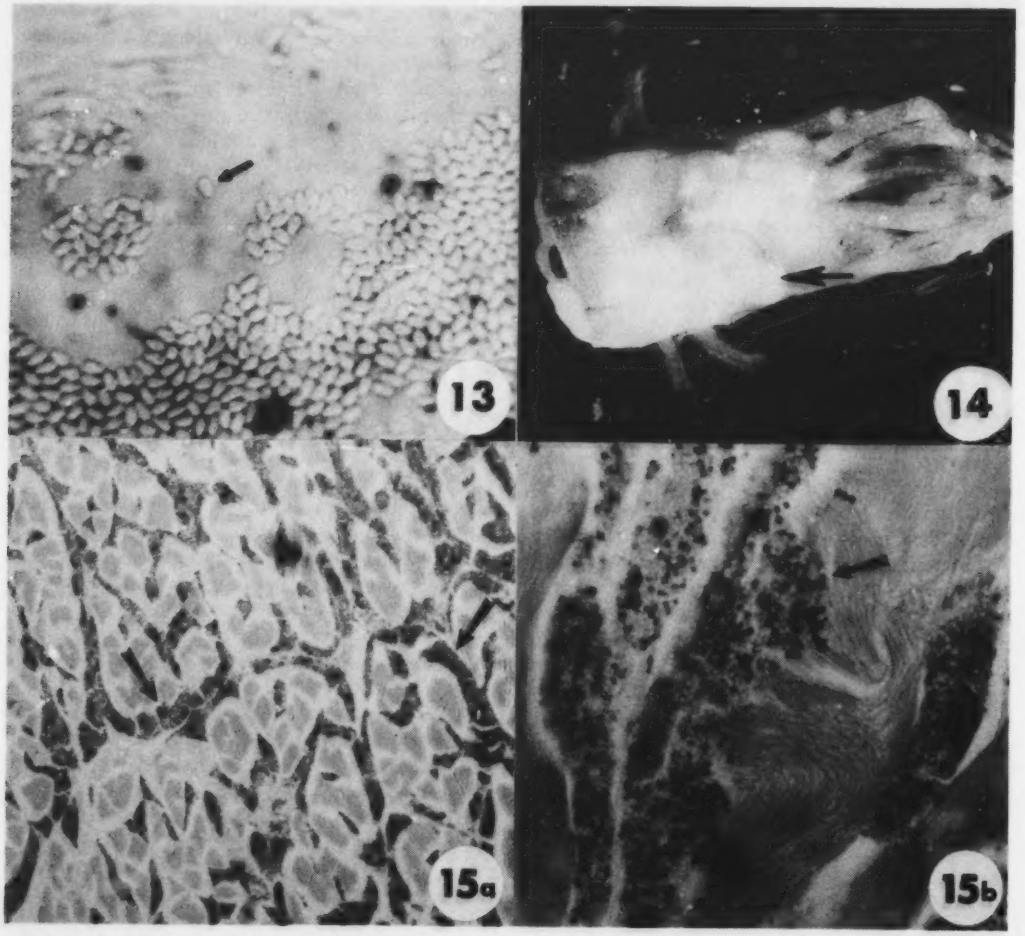


FIGURE 13.—*Nosema nelsoni* spores in fresh squash preparation of muscle from pink shrimp. ×1,500.

FIGURE 14.—White or cotton appearance of organs and muscle of penaeid shrimp infected with *Nosema nelsoni*, and *Thelohania penaei;* note opaque white appearance of gonads (arrow).

FIGURE 15.—a. Abdominal musculature heavily infected with *Nosema nelsoni;* note long spore masses between and around every muscle bundle (arrows). ×100. b. Higher magnification of spore masses of *Nosema* in histological section of muscle. ×500.

(1971), *Thelohania hunterae* (a nomen nudum) was probably *T. duorara*.

Roth and Iversen (1971) reported attempts to transmit *T. duorara* to uninfected pink shrimp in the laboratory. They were unable to do this with their method of feeding heavily infected tissue. These authors did supply some clues as to the possible modes of transmission in nature. They observed that spores of *T. duorara* found between old cuticle and new cuticle at time of molting could infect shrimp that feed on cast cuticles. Therefore, transmission could depend only on molting of the exoskeleton and not on death of the infected host.

Iversen and Kelly (1976) have reported concurrent infections of *T. duorara* and *T. penaei* in single specimens of pink shrimp.

Pleistophora (= Plistophora) penaei
Constransitch 1970

Members of this genus are characterized by sporocysts that contain 16 or more spores. Kruse

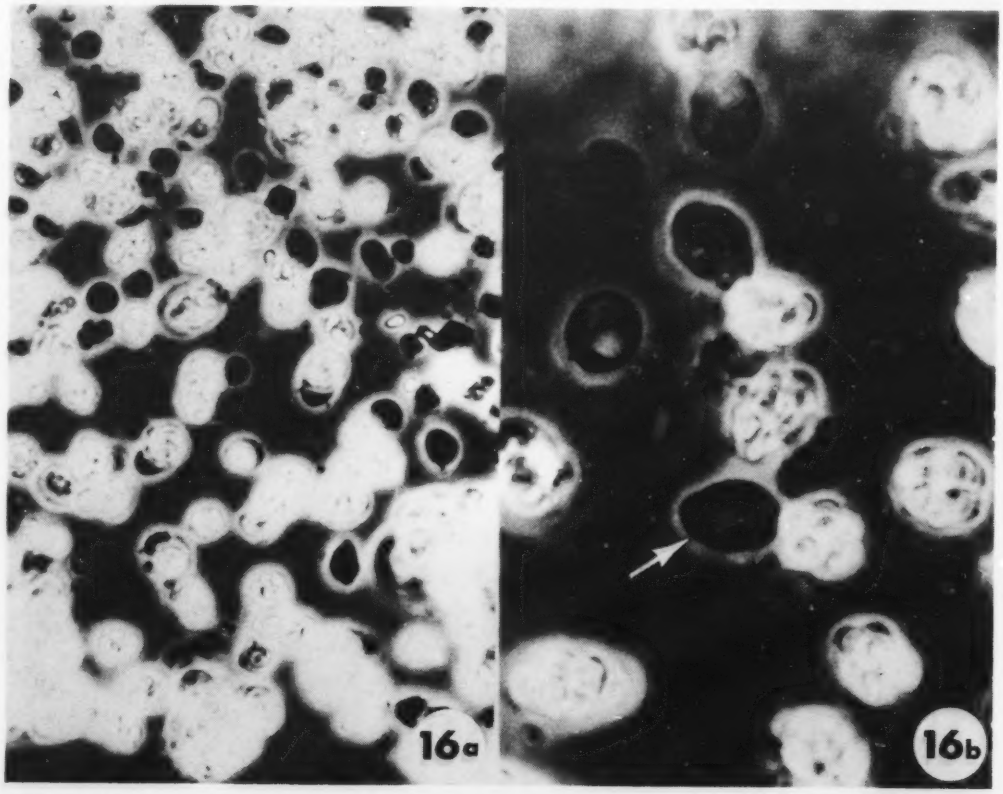


FIGURE 16.—a. *Thelohania penaei* sporocysts and spores; note approximate size of sporocysts with eight spores each; dark bodies are trophozoites or early sporonts. ×1,000. b. *Thelohania penaei* sporocysts, higher magnification; note that each sporocyst contains about eight spores; dark body (arrow) is probably an early sporont or trophozoite of this species. ×1,500.

(in Sprague 1970) first reported the genus *Pleistophora* in penaeid shrimps (*Penaeus aztecus* and *P. setiferus* from Louisiana). Constransitch (1970) named the species from Louisiana *Pleistophora penaei*. Tissues infected were tail muscle, cardiac muscle, hepatopancreas, and intestinal and stomach walls. Baxter et al. (1970) then reported a similar species from the same hosts from Texas. The Texas *Pleistophora* consisted of sporocysts that contained 40 or more spores.

Recently, Iversen and Kelly (1976) reported a *Pleistophora* sp. from the pink shrimp for the first time.

Therapeutic Measures for Microsporidosis

Very little work has been done on attempting to control or treat microsporidan infection in reared shrimp. Quick removal of "cotton" or obviously infected shrimp from tanks or ponds should aid in preventing spread of infections. Overstreet (1975) has reported some success in treating blue crabs with the drug Buquinolate to prevent infection by *Nosema michaelis*, a common microsporidan in blue crabs. He fed the drug to crabs in food contaminated with *N. michaelis* spores. He also fed the drug in food without spores 48 h preceding or following spore feeding. Control crabs were fed spores, but no drug. Drug and spore-fed blue crabs had significantly fewer infections develop than did crabs fed spores only. Whether Buquinolate or other drugs would be helpful in preventing microsporidosis in shrimp remains to be determined. Even if a drug is useful in therapy of a disease in

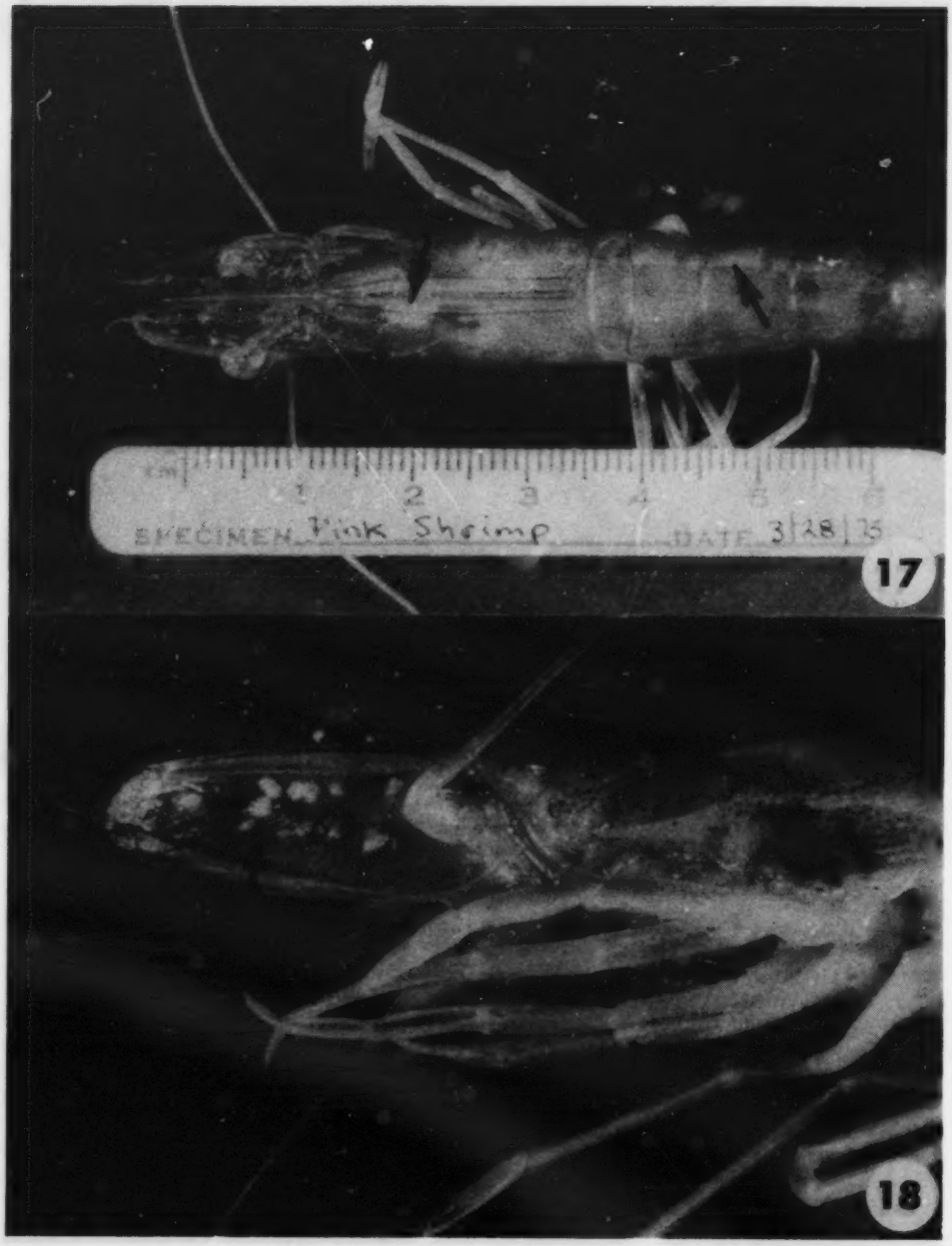


FIGURE 17.—Whole shrimp showing dorsal areas of white that indicate microsporidan infection (arrows), in this case, *Thelohania penaei*.

FIGURE 18.—White clusters of *Thelohania penaei* sporocysts in antennal scale of pink shrimp (arrow).

cultured shrimp, the problem remains for depuration of the drug from tissues prior to human consumption of the shrimp.

Subphylum Ciliophora Doflein 1901[7]

Ciliate Protozoa are very common associates of penaeids. As commensals, parasites, and pathogens, they are among the Protozoa more often encountered in or attached to penaeid shrimp. Their role, however, in the health of penaeids has not been conclusively demonstrated in most ciliate-penaeid relationships. Sprague and Couch (1971) presented a list of ciliates (and other Protozoa) found on or in decapod Crustacea. Since that report, several new finds of ciliates in penaeid shrimps have been made.

Ciliates discussed herein will be presented in order of their frequency of occurrence in penaeid shrimps (common to rare).

Class Ciliatea Perty 1852
Order Peritrichida Stein 1859
Suborder Sessilina Kahl 1933
Family Vorticellidae Ehrenberg 1838
Genus *Zoothamnium* Bory 1826
Zoothamnium sp.

An heretofore undescribed species of peritrichous ciliate, of the genus *Zoothamnium*, has been reported on penaeid shrimps along the coast of the southeastern United States Villella et al. (1970), Overstreet (1973), Johnson (1974), D. V. Lightner (pers. commun.), and I have found the colonial, stalked peritrich to be very common and frequently abundant on the gills of three commercially valuable species of penaeid shrimps.

Stalked peritrichs of the genera *Vorticella, Zoothamnium, Epistylis, Carchesium, Rhabdostyla,* and *Opisthostyla* are found attached to many hard substrates in the marine environment. The vast majority of species in these genera have not been studied, described, and named. Therefore, with this background in mind, I propose to describe, but not to formally name, the common species of *Zoothamnium* on gills and body of adults, juveniles, protozoea, and mysis of *Penaeus aztecus, P. setiferus,* and *P. duorarum*. This species will be named after further study and comparison with other species in the genus *Zoothamnium*.

Description. Vorticellid; colonial, rarely observed as individuals; 3 to 30 trophonts per colony (Figure 19); usually attached to the tips of gill filaments of hosts listed above; trophonts variable in form but usually resemble an inverted bell (45.2 μm × 33.9 μm—means of measurement of 30 individuals); with long, branching stalks (8.1 μm in diameter); phase contrast and silver-stained (protargol) specimens show that myonemes in stalks are continuous and joined, and the diameter of myonemes averages 2.0 μm (Figure 20a, b). Silver-stained specimens (Figure 20c) also reveal adoral kineties consisting of a three-component polykinety (peniculus) and a haplokinety; telotroch (Figure 21) produced by division of stalked trophont, slightly smaller than stalked trophont; lifecycle direct, that is, the telotroch may swim free of mother colony and attach to surface of gill or body of shrimp, secrete a stalk, and become progenitor of a colony; sexual reproductive cycle not observed for this species, but probably is a conjugative process as in other peritrichs having microconjugants and macroconjugants. I have observed only pairs and small colonies (3, 4 trophonts) of *Zoothamnium* sp. attached to body surfaces of larval (mysis and protozoea) brown shrimp.

Overstreet (1973) gave extensive data on the frequency of occurrence of *Zoothamnium* on penaeid shrimps. He found that an increase in density of hosts held in captivity was paralleled by an increase in density of peritrichs on gills. This is similar to what Couch (1971) observed for blue crabs infested with *Lagenophrys callinectes* Couch (1967), a gill peritrich. Overstreet (1973) also was able to correlate, positively in one test, increased mortality in shrimp with heavy infestations by *Zoothamnium* on their gills. However, he was not convinced that the correlation was valid. More extensive work on this relationship is needed.

The mechanism of injury to penaeids infested with peritrichous ciliates would probably be oxygen starvation or asphyxiation due to blockage of gas exchange at the gill surface. The attachment stalk of *Zoothamnium* sp. does not penetrate the cuticle of shrimp.

Family Lagenophryidae Kahl 1935
Genus *Lagenophrys* Stein 1852
Lagenophry lunatus Imamura 1940

A species of *Lagenophrys* was reported from the cuticle of *Penaeus setiferus* by Johnson (1974) and

[7]Most ciliatologists and many protozoologists now consider the Ciliophora as a phylum, but herein the Honigberg et al. (1964) classification scheme is followed.

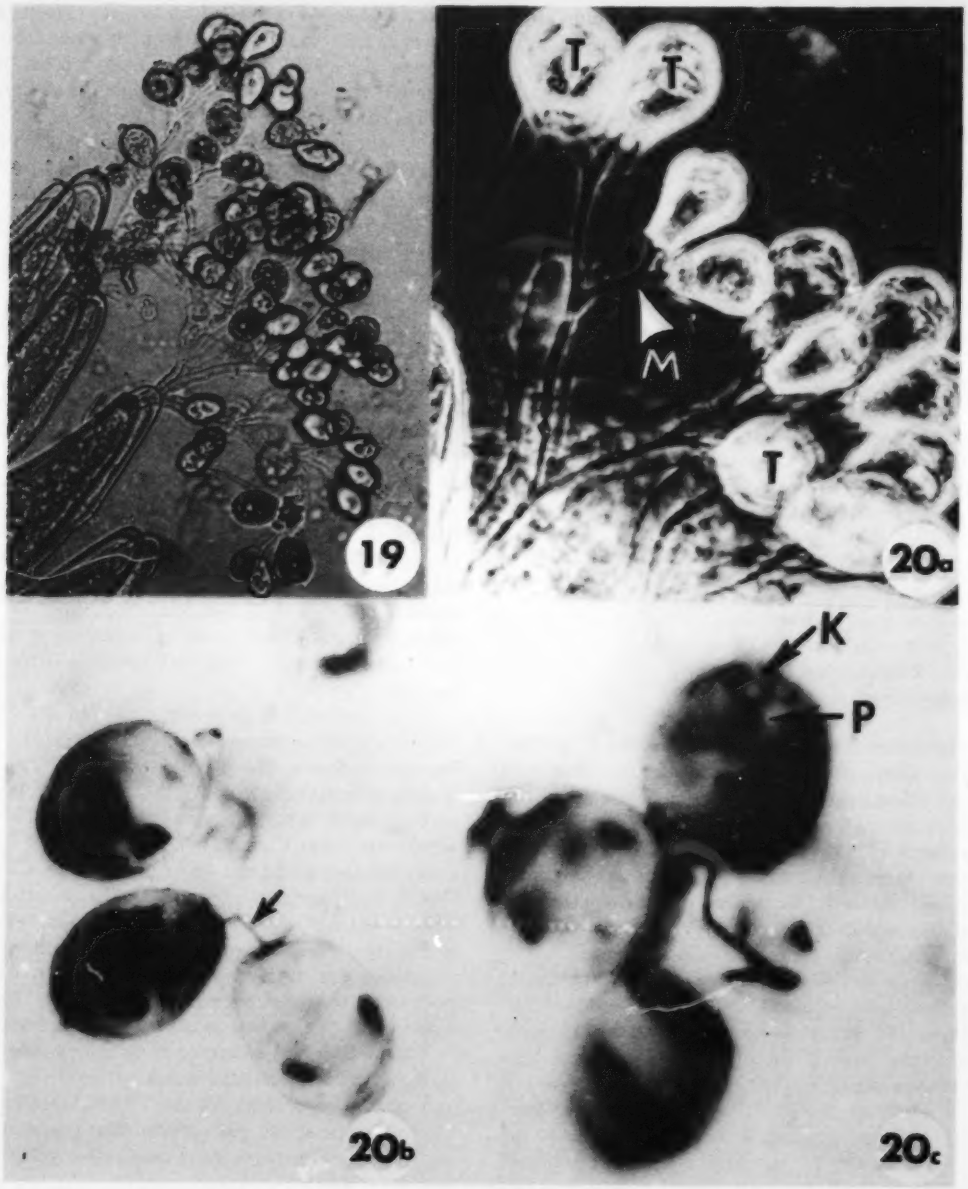


FIGURE 19.—Colonies of *Zoothamnium* sp. attached to end of gill filaments in pink shrimp; this represents a light infestation; heavy infections would cover all filaments. ×200.

FIGURE 20.—a. Phase contrast photomicrograph of *Zoothamnium* colony showing stalk myonemes (M) that are continuous with one another, the major distinguishing characteristic of the genus; note inverted bell shape of contracted trophonts (T) and thickness of stalk sheath that surround myonemes (arrow). ×500. b. Protargol treated specimens of *Zoothamnium;* note beltlike, horseshoe-shaped macronucleus and Protargol-positive myonemes of stalk (arrow). ×1,200. c. Protargol-treated *Zoothamnium;* trophont (in focus) shows peniculus (P) in infundibulum (arrow); note pattern of kineties (K) making up peniculus. ×1,200.

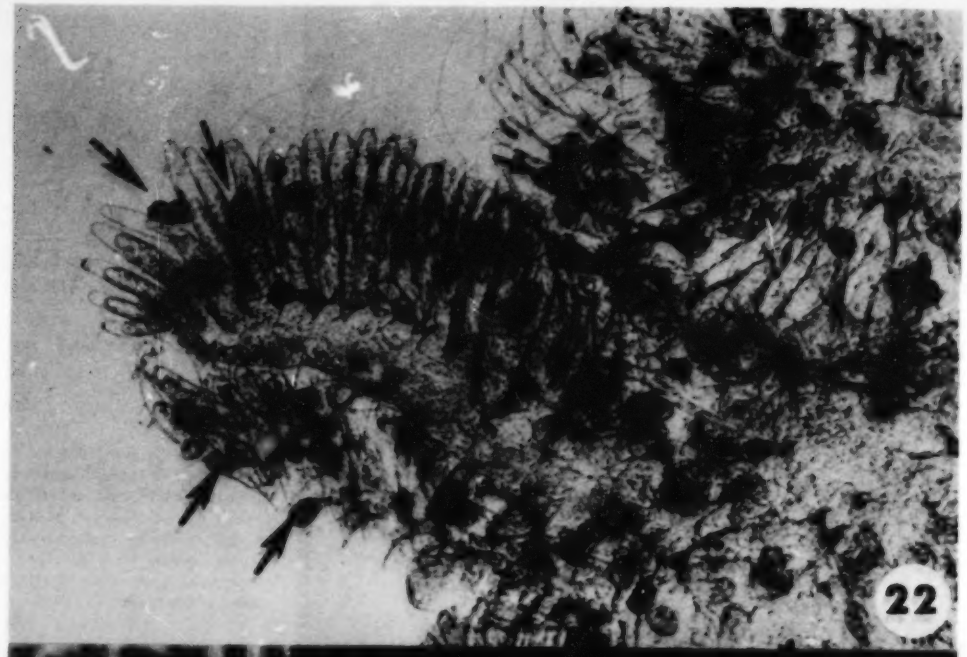
FIGURE 21.—Telotroch stage (arrow) of *Zoothamnium* produced from division of trophont (phase contrast); this is the dispersal stage for the species; the telotroch is motile and possesses a ventral girdle of cilia. Note the trophont at upper right with extended adoral ciliature (arrow). ×500.

by Lightner (1975) in Texas. From a photomicrograph kindly loaned to me by Johnson, I have tentatively identified this loricate peritrich as *Lagenophrys lunatus*. This species is commonly found on the cuticle of paleomonid shrimps along the east coast and gulf coast of the United States, but Johnson's report, if accurate, is the first for a penaeid. It is possible that the species of shrimp examined by Johnson was a grass shrimp, *Paleomonetes* sp. Species of *Lagenophrys* are usually host specific, and though I have examined many penaeid shrimps, I have not observed *Lagenophrys* sp. on any. Couch (1971) gave a detailed discussion of the possible effects of *Lagenophrys* spp. on the cuticles and gills of decapod Crustacea with particular reference to *L. callinectes* on the gills of the blue crab, *Callinectes sapidus*. Erosion of cuticle surface and interference with gas exchange at the gill surface in heavy infestations are possible effects of *Lagenophrys*.

Order Apostomatida Chatton and Lwoff 1928
Family Foettingeriidae Chatton 1911
Genus Uncertain

The encysted form (phoront) of an undescribed apostome ciliate has been observed on the gills of *Penaeus duorarum* (Figures 22, 23) in northwest Florida. The cysts are decumbent, ellipsoidal bodies that are 41 μm wide by 60 μm long (range: 20.7-41.4 μm by 27.6-60.0 μm). The cyst wall is from 1 to 3 μm thick and is semitransparent.

Heavy infestations of this ciliate occur on gills of pink shrimp during periods of warm to moderately cool weather when shrimp are held under crowded conditions (Figure 22). The cysts are most often attached to the gills at the point of branching of the distal processes variously termed lamellae, filaments, or tertiary structures (Figure 22). The lifecycle of this ciliate has not been elucidated, and it cannot be assigned to a genus until silver-



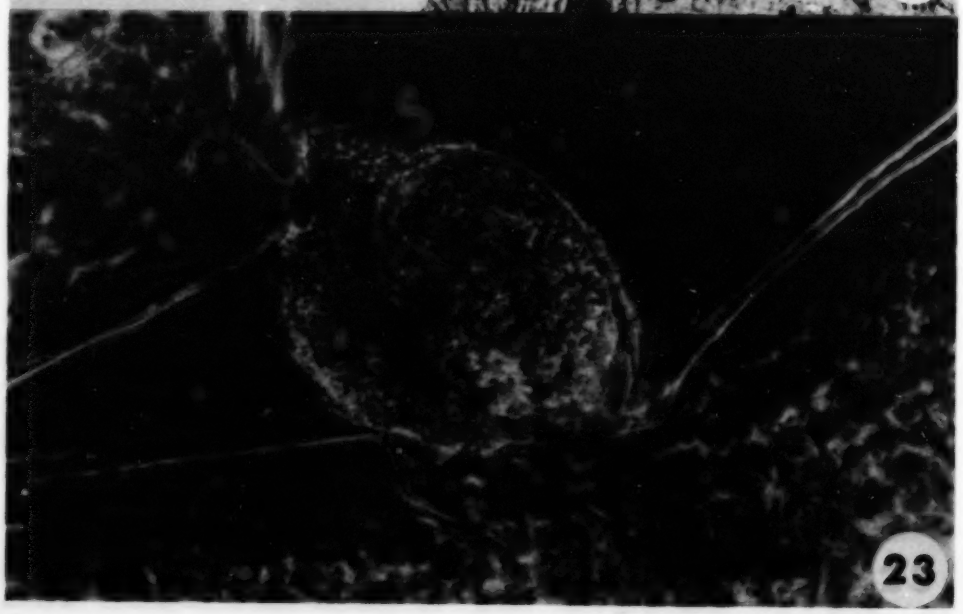


FIGURE 22.—Cysts (phoront) of apostome ciliate (arrows) on gills of pink shrimp; this is a moderately heavy infestation. ×150.
FIGURE 23.—Single cyst (phoront) of unidentified apostome attached near base of gill filament; note ellipsoid form. ×1,000.

staining studies and lifecycle studies are more complete.

Reports by Chatton (1911), Chatton and Lwoff (1935), Debaisieux (1960), and Bradbury (1966, 1973) have demonstrated the common occurrence of apostomes on Crustacea that occupy ecological niches near that of the pink shrimp. The present species has not been found associated with mortality in shrimp, although severe infestations may cover much gill surface and blackened areas of infested gills are found. Species of two known apostome genera, *Synophyra* and *Terebrospira*, cause considerable damage by penetrating the cuticle of their crustacean hosts (Chatton and Lwoff 1926; Bradbury 1974).

R. M. Overstreet (pers. commun.) has found similar cysts on gills of brown and white shrimp, and Feigenbaum (1973) reported cysts similar to those described above on gills of *Penaeus brasiliensis* from Biscayne Bay. The cysts of apostomes could be confused with the loricae of species of *Lagenophrys*, Care should be taken to distinguish them. Loricae of *Lagenophrys* spp. have apertures surrounded by liplike structures (Couch 1973).

Order Scuticociliatida Small 1967
Genus *Parauronema* Thompson 1967
Parauronema sp.

An undescribed species of ciliate was observed in the hemocoel of protozoeal, mysid, and juvenile stages of living, moribund, and dead brown shrimp from a mass mortality which occurred at a commercial shrimp hatchery[8] during April 1974. In a sample of 139 larvae examined, 28.8% were infected by the ciliate (Tables 1, 2). The ciliate is ovoid to pyriform in shape, ranging in length from 23.6 to 31.6 μm, and in width from 9.2 to 12.2 μm (Figures 24, 25). It has a uniform body ciliature originating from longitudinal kineties (Figure 25) as revealed by Protargol silver staining.

The ciliate was observed swimming about in hemolymph of infected shrimp larvae and juveniles. Often the affected shrimp were still alive and active, but several that were dead or quite moribund contained ciliates. John Corliss (University of Maryland) tentatively identified the ciliate as a species of *Parauronema*. More studies are required in order to name this ciliate.

[8]Mortality was that reported on preceding pages (under virus section). Several microorganisms were associated with this mortality.

Apparently the ciliate causes mechanical injury in infected shrimp by replacing and dislodging tissues. I have been unable to determine from limited observations whether or not the ciliate is histophagous. In some shrimp the ciliates were numerous enough to fill the entire hemocoel and abdomen. The fact that living shrimp larvae were infected by the ciliates strongly suggests that the ciliate probably contributes to pathogenesis and mortality and that it is an opportunistic invader following initial breaks in the host's defense mechanisms due, possibly, to the presence of other pathogenic microorganisms (the *Baculovirus* and a flagellate to be described next). Tables 1 and 2 show the relationship of prevalence of ciliate with virus and flagellate in a sample of young brown shrimp from a stock suffering mortality.

Subclass Suctoria Haeckel 1866
Order Suctorida Claparede and Lachmann 1858
Family Ephelotidae Kent 1881
Genus *Ephelota* Wright 1858
Ephelota sp.

Protozoeal and mysid stages of brown shrimp were found infested on a single occasion with an undescribed species of *Ephelota*. The larval shrimp were examined in March. Each larva had from one to seven individual *Ephelota* sp. attached to their cuticles usually on the pleural plates or on the telson. The suctorian possesses a characteristically striated attachment stalk and a trophont with both suctorial and prehensile tentacles. These Protozoa were not abundant enough to cause embarrassment to the larval shrimp.

Subphylum Sarcomastigophora
Honigberg and Balamuth 1963
Class Zoomastigophorea Calkins 1909
Order Kinetoplastida Honigberg 1963
Suborder Trypanosomatina Kent 1880
Family Trypanosomatidae Doflein 1901
Genus *Leptomonas* Kent 1880
Leptomonas sp.

An undescribed species of flagellate was associated with the mass mortality of brown shrimp larvae (see *Baculovirus* and *Parauronema* sections) (Figure 26). This form is tentatively assigned to the genus *Leptomonas* based on subsequently described characteristics. The flagellate was studied alive (bright field and phase contrast), fixed, and stained with Harris' hematoxylin and

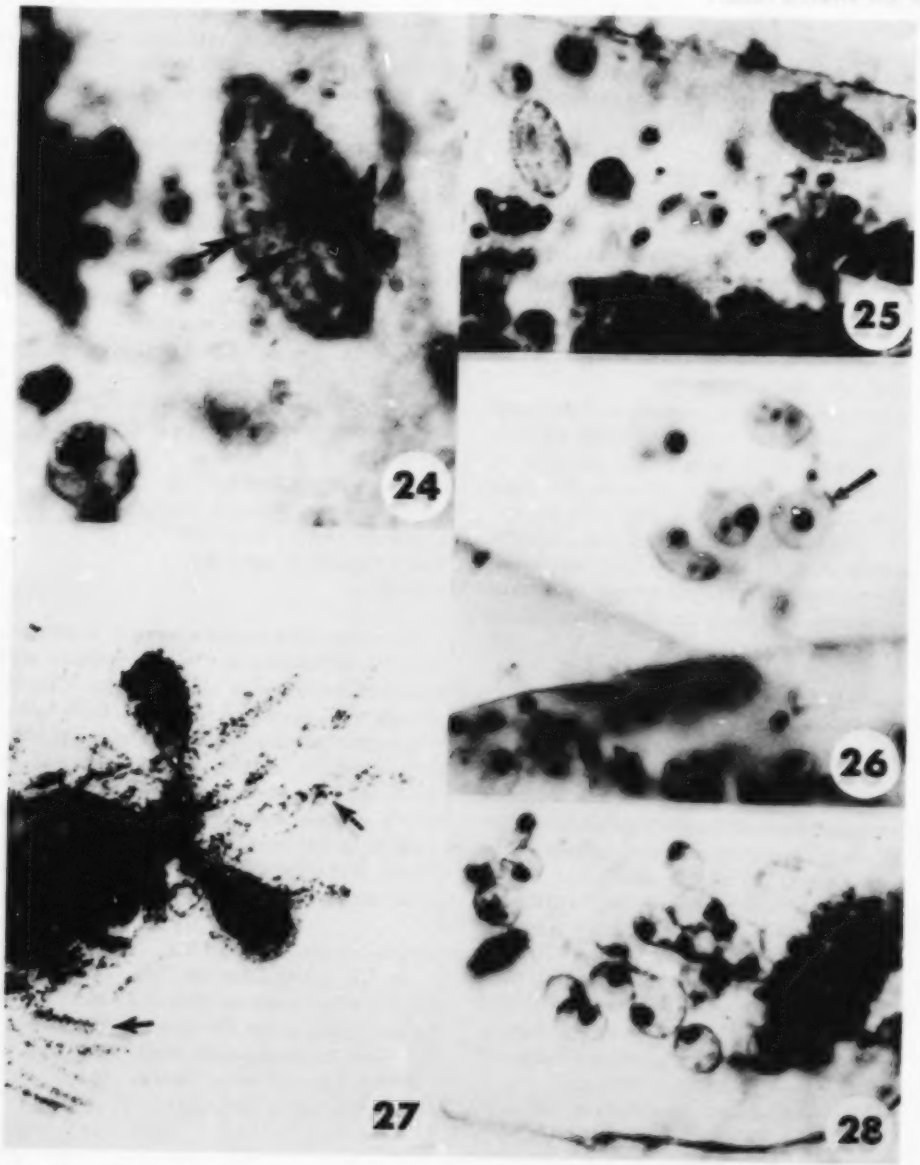


FIGURE 24.—Trophont of ciliate, *Parauronema* sp., in hemocoel of brown shrimp larva; note body form and longitudinal rows of kinetosomes on body surface (arrows) (Protargol). ×1,300.

FIGURE 25.—Two trophs of *Parauronema* sp. in body of brown shrimp larva; in living shrimp these ciliates swim about in hemolymph. ×900.

FIGURE 26.—Cells of *Leptomonas* sp., a flagellate, from hemolymph space in appendage of larval brown shrimp; note flagellar base as revealed by Protargol stain (arrow); compact nucleus is also visible. ×1,000.

FIGURE 27.—Head and anterior appendages of larval brown shrimp heavily infected with *Leptomonas* sp.; note antennae, antennules, and thoracic legs filled with flagellate (arrows).

FIGURE 28.—Cystlike stages of *Leptomonas* in hemocoel of larval brown shrimp (Protargol). ×1,000.

Protargol silver protein. It is the first flagellate reported to be associated with shrimp mortalities. The flagellate occurred in the hemocoel, abdomen, and all appendages of protozoel and mysid stages of brown shrimp during April 1974 (Figure 27). The flagellate was found in 64% of larvae examined from the mortality; living, moribund, and dead larvae were infected (Tables 1, 2).

The flagellates were variable in form ranging from 7.8 to 11.7 μm with an average diameter of 9.4 μm. A compact nucleus (2 or 3 μm) containing a large endosome was situated medianly. The cytoplasm ranged from clear to opaque and often contained various inclusions. In life, the flagellate was slightly pyriform with a terminal, single flagellum (Figure 29). Specimens stained with protargol clearly demonstrated a flagellar base, parabasal body, or blepharoplast (karyomastigont) (Figures 26, 29). A possible cyst stage (7-9 μm) was observed in advanced or heavy infections in the hemocoel (Figures 28, 29f). Dividing stages, observed occasionally, contained nuclei undergoing division without loss of nuclear membranes (Figures 29e).

The role, if any, that *Leptomonas* sp. plays in the mortality of shrimp larvae is unknown. Other than mechanical damage, there appears to be little evidence of a pathogenic mechanism for the flagellate. It is possible that the flagellate is a secondary invader of a weakened host, possibly from encysted forms which may exist in the hindgut of the host.

Platyhelminthes

Flatworms have been described as parasites of all commercial species of penaeid shrimps in the United States. These include digenetic trematodes

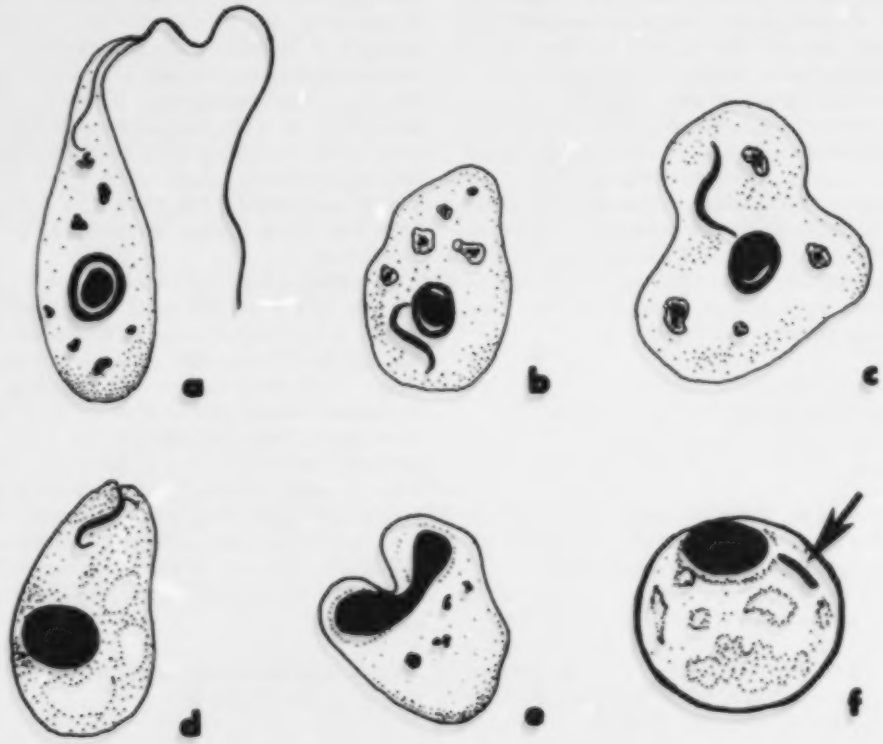


FIGURE 29.—a. *Leptomonas* sp. drawn from life with flagellum. b, c, d. Forms of the flagellate (possibly amastigote stages) as they appear in Protargol-stained body (hemolymph) of brown shrimp. e. Cell division in flagellate showing karyokinesis and longitudinal cytoplasmic fission. f. Possible cyst stage *Leptomonas* from hemocoel of larval shrimp. Note Protargol-positive kinetoplast near nucleus (arrow points to kinetoplast). (All figures ×2,900.)

and cestodes. The role of these worms as agents of disease in shrimps is uncertain. Most of the species reported, to date, appear to have little effect on individual shrimp infested, and probably little significant effect on populations of penaeids. However, flatworms in penaeid shrimps are often conspicuous and, thus, attract considerable attention. Penaeid shrimp usually play the role of intermediate host for most, if not all, flatworms they harbor; therefore, shrimps play a significant role in the ecology of parasites that may be transmitted through the food web to higher vertebrate hosts.

Class Trematoda Rudolphi 1808
Subclass Digenea Carus 1863
Family Microphallidae (Travassos 1920)
Genus *Microphallus* Ward 1901
Microphallus sp.

Hutton et al. (1959) reported an undescribed species of microphallid trematode metacercariae from pink shrimp. They found that from two to three metacercarial cysts up to hundreds (from 1.2 to 1.5 mm in diameter) were encysted in muscle tissue surrounding internal organs, particularly the cephalothoracic and abdominal musculature. No effect on the shrimp host was reported.

Overstreet (1973) also reported an unidentified microphallid metacercaria from abdominal muscles of white shrimp from Barataria Bay, La. The cysts were 93-95 μm to 77-83 μm, much smaller than those reported from pink shrimp from west Florida by Hutton et al. (1959).

Family Opecoelidae Ozaki 1925
Genus *Opecoeloides* (Odhner 1928)
Opecoeloides fimbriatus (Linton 1934)
Sogandares-Bernal and Hutton 1959

Metacercariae of this trematode (Figure 30) encyst in hepatopancreas, other internal organs, and beneath the exoskeleton of *Penaeus duorarum*, *P. setiferus*, and *P. aztecus*. This is a very common parasite of penaeids, occurring in up to 90% of some samples of pink shrimp taken during the summer from Apalachee Bay, Fla. No extreme pathogenesis in shrimp has been reported associated with *O. fimbriatus*. The worm is approximately 1.5 to 2.0 mm long when excysted and is quickly identified by its possession of an extremely pedunculate acetabulum (Figure 30). The sexually mature worm (adult) is found mostly in fishes of the family Sciaenidae which feed on shrimps. The metacercaria is found in penaeids from the Gulf and Georgia coasts.

Class Cestoidea Rudolphi 1809
Order Trypanorhyncha Diesing 1863
Family Eutetrarhynchidae Guiart 1927
Genus *Prochristianella* Dolfus 1946
Prochristianella hispida (Linton 1890)
Campbell and Carvajal 1975
Synonyms: *Rhynchobothrium hispidum* Linton 1890; *P. penaei* Kruse 1959

Plerocercoid larvae of this tapeworm are very common in *Penaeus setiferus*, *P. duorarum*, and *P. aztecus*. I have found up to 95% of large samples of *P. duorarum* from northwest Florida to harbor the cestode. This cestode is found mainly in the hepatopancreas of the host (Figure 31), and most often fails to elicit any strong pathologic response from the shrimp. Sparks and Fontaine (1973) and Feigenbaum and Carnuccio (1976) reported a strong host reponse to the plerocercoid when it encysted in hepatopancreas. I have not observed this in several hundred hosts examined, but host destruction of trypanorhynchan plerocerci may occur rarely in shrimp. Most evidence suggests a long and relatively tolerant relationship between shrimp and cestode. Often a single shrimp will have one to two dozen encysted larvae in its hepatopancreas.

According to my measurements, the worm (Figure 32a, b) has the following mean dimensions: length = 1.12 mm; bladder or blastocyst = 0.58 mm long by 0.37 mm wide; and scolex (below bothridia) = 0.11 mm wide by 0.35 mm long. These measurements are close to those of Kruse's (1959) description. Though no lifecycle has been experimentally completed for a trypanorhynchan, the hosts for adult worms of this group are probably sharks and rays. From nature, cestodes of this order have been found in the spiral valves of elasmobranchii (Kruse 1959). Aldrich (1965) and Ragan and Aldrich (1972) gave host-parasite data on this species.

Parachristianella monomegacantha Kruse 1959
P. dimegacantha Kruse 1959

Kruse (1959) described two other trypanorhynchan plerocercoid larvae from *Penaeus duorarum*. These species were found in the hepatopancreas of shrimp from the northern gulf coast and are distinct from one another "in hook arrangement and

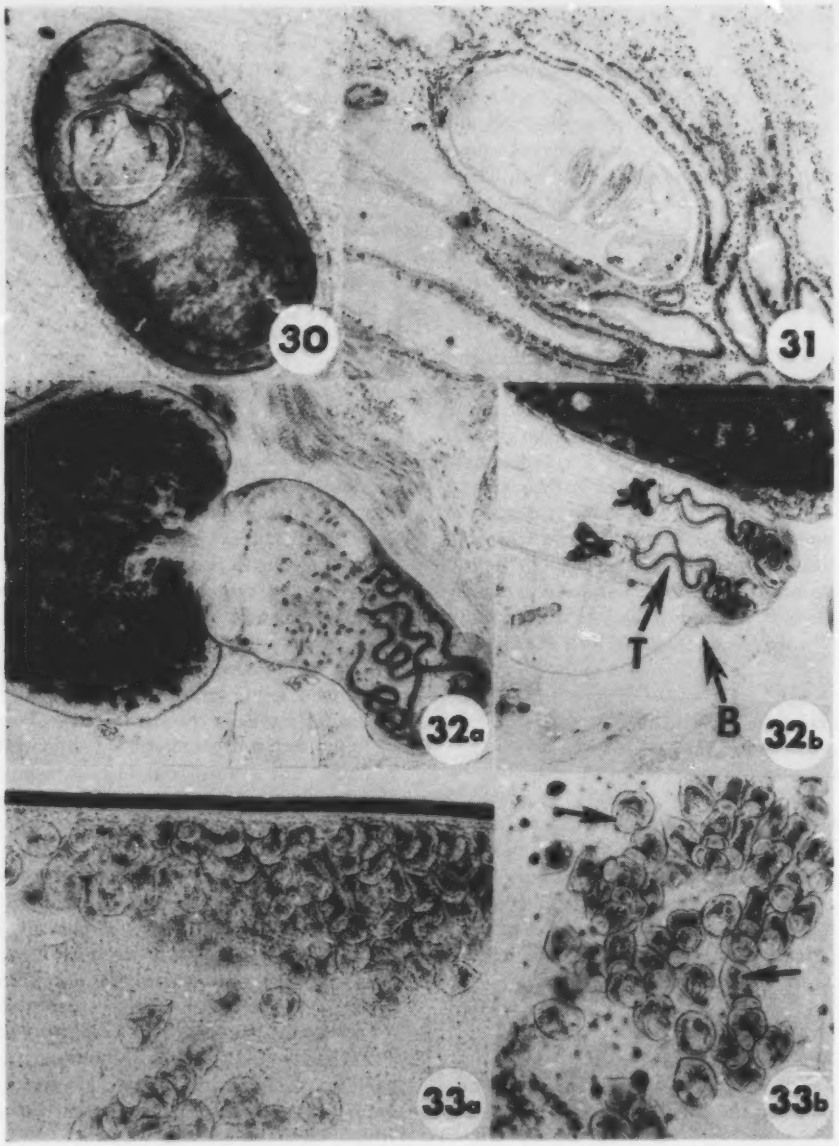


FIGURE 30.—Metacercaria of *Opecoeloides fimbriatus*, digenetic trematode, from hepatopancreas or hemocoel of adult pink shrimp. This species is quickly identified by its large, pedunculate acetabulum (arrow). ×70.

FIGURE 31.—Section of plerocercoid larva of *Prochristianella hispida* encysted in hepatopancreas of pink shrimp; note cyst wall and lack of host cellular response (Feulgen picro-methyl blue stain). ×50.

FIGURE 32.—a. Fresh wet mount of plerocercus of *P. hispida;* note scolex and blastocyst. ×50. b. Scolex of *P. hispida;* note tentacles (T) and bothria (B) (arrows). ×50.

FIGURE 33.—a. Larvae of an unidentified cestode commonly found in hemocoel of penaeid shrimps; this figure shows a mass of larvae against the midgut lining (dark line). ×25. b. Unidentified cestode larvae showing calcareous corpuscles and large sucker (arrows). ×25.

in the relative sizes of their bothridia, bulbs, and post-bulbosal regions."

The genus differs from *Prochristianella* in the morphology of the blastocyst; species of the latter genus having a division between anterior and posterior portions, with large granules contained in the anterior division of the blastocyst. These worms apparently do not harm their hosts significantly.

Parachristianella heteromegacanthus
Feigenbaum 1975

The most recent species to be described is from *Penaeus brasiliensis* from Biscayne Bay. Twenty percent of this shrimp were infected with fewer than 1.5 worms occurring in each infected shrimp. Corkern (1970) found an average of 2.3 specimens of *P. dimegacantha* per infected brown shrimp from Galveston Bay, Tex. Prevalence data from Corkern's work shows 23% brown shrimp infected, a figure close to that of Feigenbaum's (1975) 20% for *P. heteromegacanthus*. Tentacle hook arrangements in *P. heteromegacantha* differed from those in *P. monomegacantha* and *P. dimegacantha*.

Family Renibulbidae Feigenbaum 1975
Genus *Renibulbus* Feigenbaum 1975
Renibulbus penaeus Feigenbaum 1975

To date, this species was found in 14.3% of *Penaeus brasiliensis* examined from Biscayne Bay. The short kidney-shaped bulbs in the scolex of this cestode set it apart from other trypanorhynchan cestodes in penaeid hosts. No organ site of infection was given by Feigenbaum (1975) for this worm, and no pathogenesis was indicated.

Unknown Cestode Larva

Hutton et al. (1959), Kruse (1959), Overstreet (1973), Feigenbaum (1975), and I have found a small pyriform cestode larval stage (Figure 33a, b) commonly in the intestine of penaeid shrimps from the Gulf and Atlantic coasts of Florida. This worm also is found in large numbers in several tissues of infected shrimp, namely, the muscles and hemocoel. The worm possesses a large anterior sucker and many refringent calcareous corpuscles in its body, and is approximately 0.61 to 0.81 mm long by 0.12 to 0.22 mm wide. Large numbers of this worm may occlude the intestinal lumen or cause perforation of the intestinal wall. Several hundred larvae have been counted in a single shrimp. Hosts, to date, include *Penaeus duorarum, P. aztecus, P. setiferus,* and *P. brasiliensis*.

Nematodes

Phylum Aschelminthes Grobben 1910
Class Nematoda (Rudolphi 1809) Cobb 1919
Superfamily Ascaridoidea
(Railliet and Henry 1915)
Genus *Thynnascaris* Dolfus 1933
Thynnascaris sp.

Overstreet (1973) reported that the nematode larvae identified by Kruse (1959), Hutton et al. (1959), and Corkern (1970) as *Contracaecum* sp. in penaeid shrimps should be considered species of *Thynnascaris*. Norris and Overstreet (1976) have found that at least two species occur in penaeid shrimps in North America. Characteristics of this genus are short intestinal caecum and longer ventricular appendix combined with the position of the excretory pore near the nerve ring. Figures 34 and 35 are photomicrographs of *Thynnascaris* sp. recovered from hepatopancreas and cephalothorax of *Penaeus duorarum* near Pensacola. I have not found it commonly in shrimp from west Florida, but Overstreet (1973) reported that Donald Norris of his laboratory found up to 31% of white and brown shrimp from Mississippi Sound and adjacent waters infected during summer months. *Thynnascaris* sp. juveniles measure 1.02 to 2.40 mm long by 0.06 to 0.10 mm wide.

Overstreet (1973) reported two specimens of *Spirocamallanus pereirai* Olsen 1952, in the intestine of *Penaeus setiferus* from near Biloxi, Miss. These were third stage larval nematodes which measured 1.00 mm long by 0.03 mm wide. Overstreet suggested that the shrimp may serve as a paratenic host and that copepods may serve as a more common source or vector for this nematode which normally matures in fishes.

Several species of free-living nematodes, commonly found in shrimp habitat, have been reported as facultative commensals or inquilines of penaeids. Shrimp may take these worms in larval stages when they feed on detritus or bottom organisms in nature or in artificial ponds. Specimens of *Leptolaimus* sp. and *Croconema* sp. have been found by Overstreet (1973) in brown and white shrimps from Mississippi. Other than phys-

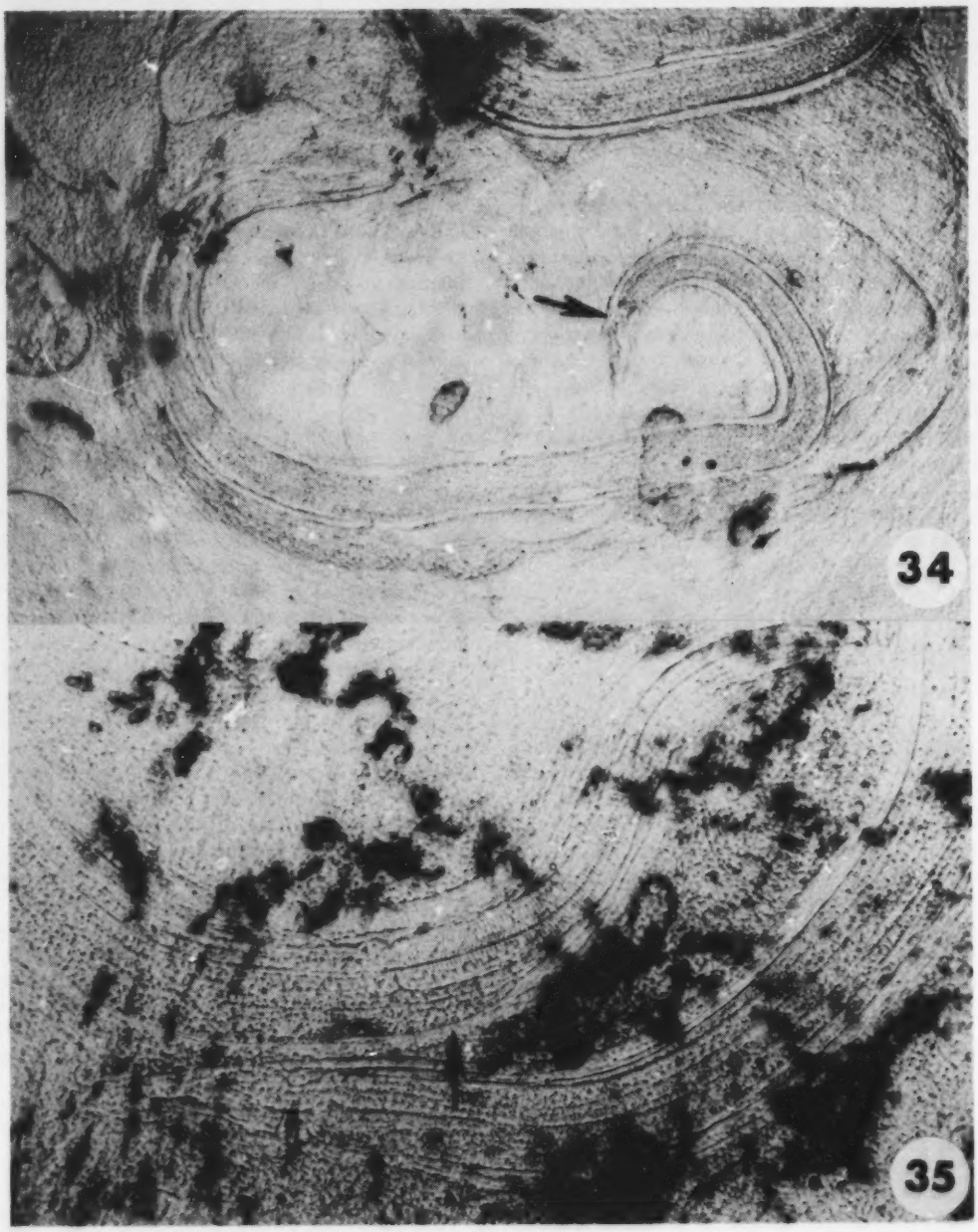


FIGURE 34.—*Thynnascaris* sp. larvae in tissue squash from pink shrimp. Whole worm larva in view; note cellular arrangement at posterior of worm (arrow). ×50.

FIGURE 35.—Higher magnification of *Thynnascaris* sp.; note the intestinal caecum that turns anterior from the intestine (arrow). ×100.

ical disruption of tissues, no mechanism of pathogenesis is apparent for nematodes in shrimp.

NONINFECTIOUS DISEASES

Toxic Responses

In the last decade, because of interest in aquatic pollution, some research has been done on toxic responses of penaeid shrimps to a variety of chemicals and heavy metals. Most of this work has been done in pollution-oriented laboratories; however, few attempts have been made to apply results to interpretation of field conditions. Results obtained have been reported mostly as toxicity of specific chemical agents in terms of short-term lethality or longer-term mortality. Unfortunately, little indicative cellular or tissue changes caused by toxicants has been described for penaeid shrimps. I shall divide this section into categories of toxicants that have been tested or studied in penaeids. The following categories will be covered: organochlorines, organophosphates, carbamates, oil or petroleum products, heavy metals, and chemotherapeutic chemicals.

Organochlorines

Since World War II many kinds of pesticides and industrial chemicals containing or consisting of chlorinated hydrocarbons have been inadvertently or intentionally released into the environment. Aquatic life is exposed to these compounds because the aquatic portion of the biosphere often behaves as a "sink" or receptacle for these compounds due to runoff or fallout. Some of these compounds or their metabolites are refractory to breakdown, and thus tend to accumulate in various compartments of the aquatic environment. Experimental shrimp have been found to accumulate certain chlorinated compounds in the laboratory and feral shrimp have possessed detectable levels when taken directly from contaminated or apparently "clean" waters. Jack Lowe of the USEPA Laboratory, Gulf Breeze, has found, over several years of testing, that penaeid shrimps generally are far more sensitive to toxic effects of most insecticides than are fishes or mollusks (Table 4). The effects of some of the better known compounds will be reviewed here.

TABLE 4.—Comparative toxicity of pesticides to three estuarine taxa—most sensitive (1) to least sensitive (3).[1]

Pesticide	Penaeid shrimp	Fish	Oysters
Chlordane	1	2	3
DDT	1	2	3
Dieldrin	1	3	2
Endrin	1	2	3
Heptachlor	1	3	2
Toxaphene	2	1	3
Guthion	1	2	3
Malathion	1	2	3
Parathion	1	2	3
Carbaryl	1	2	3
Carbofuran	1	2	3
2,4-D (BEE)	3	2	3
Atrazine	1	2	3
Du-ter	3	2	1
Difolatan	3	2	1

[1] This table was prepared by Jack I. Lowe who graciously granted permission for its use here. The table has not been published previously.

DDT

White shrimp, which died as a result of DDT exposure, accumulated up to 40.40 ppm DDT and DDE in hepatopancreas after 18 days exposure to 0.20 ppb in flowing seawater (Nimmo et al. 1970). Exposure to DDT concentrations greater than 0.10 ppb was lethal to pink shrimp in 28 days. A physiological effect of DDT exposure in pink and brown shrimps was loss of certain cations in the hepatopancreas (Nimmo and Blackman 1972). Sodium and potassium concentrations in shrimp exposed to 0.05 ppb DDT for 20 days were lower than in those not exposed. Magnesium, however, was not significantly lowered. The significance of reduced cations in the hepatopancreas of shrimp for the pathophysiological behavior of shrimp is not known. Blood protein levels also have been found to drop in shrimp exposed to DDT. There are no reports of histopathological changes in penaeids following exposure to DDT. In acute, high-concentration exposures, shrimp showed tremors, hyperkinetic behavior, and paralysis, classic signs of DDT poisoning in arthropods. After extended exposure to low concentrations of DDT, shrimp did not become paralyzed, but sank into lethargy, refused food, and then died.

Dieldrin

Pink shrimp were more sensitive to dieldrin than were grass shrimp in test exposures. However, both species died when exposed to concentrations of dieldrin in the low parts-per-billion range. Pink shrimp had a 96-h LC_{50} of 0.9 ppb dieldrin (Parrish et al. 1973). No histopathological effects of dieldrin in penaeid shrimps have been reported.

Mirex

Juvenile pink and brown shrimps died after exposure to low concentrations of mirex. Twenty-five percent of a sample of pink shrimp died during 7 days exposure to 1.0 ppb mirex. However, all survivors from this test died after 4 days in mirex-free seawater, demonstrating a delayed toxic effect of mirex (Lowe et al. 1971).

I have examined both shrimp and blue crabs exposed to low concentrations of mirex for long periods (30 days or more) for histopathological effects. No pathologic effects at the tissue level were found in the animals which I examined. Organs studied were muscle, hepatopancreas, and gonads.

PCB's (Polychlorinated Biphenyls)

These industrial chemicals have been at large in the aquatic environment for many years due to leakage from water and waste effluents, disposal of dielectric fluids, and other industrial sources (Broadhurst 1972). It is a well-established fact that certain fresh and marine bodies of water are contaminated with various compounds of PCB (Sodergren et al. 1972; Nimmo, Blackman, Wilson, and Forester 1971; Nimmo, Wilson, Blackman, and Wilson 1971; Nimmo et al. 1975). As recently as 1970, Duke et al. reported PCB, Aroclor 1254, in water, sediments, and tissue of animals (including penaeid shrimps) from Escambia Bay, near Pensacola.

At the U.S. Environmental Protection Agency Laboratory (Gulf Breeze, Fla.), much research has been done on the effects of PCB's on estuarine species with emphasis on pink and brown shrimps. These two penaeids were killed in 2-wk exposures to 0.9, 1.4, and 4.0 ppb Aroclor 1254 in flowing seawater. The minimum level causing mortality was 0.9 ppb. Penaeid shrimps appeared to suffer greatest mortality when exposed during premolt (just before molting) and during molt. Most exposed shrimp became lethargic, stopped feeding, and did not dig into the substrate (digging is a normal activity for penaeids). Subtle to dramatic chromatophore changes in the cuticle of exposed shrimp were more frequent and obvious than in control shrimp.

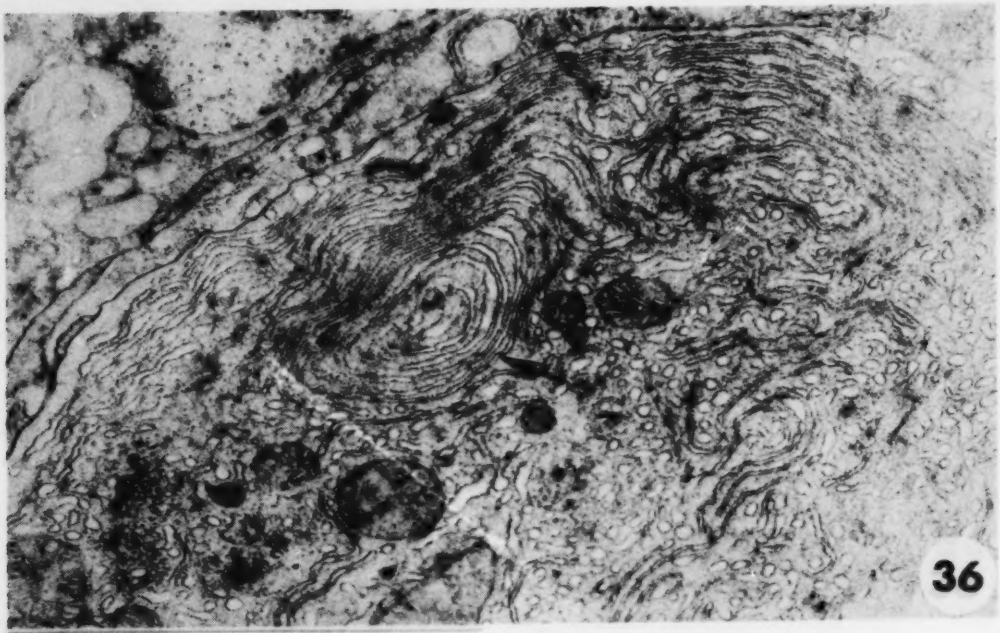
On the light microscopical level, no lesions were consistently found that were indicative of PCB exposure in shrimp (Couch and Nimmo 1974a). However, several interesting cytopathic changes were noted in exposed shrimp studied with EM.

Pink shrimp were exposed to 3 ppb Aroclor 1254 in flowing seawater for 30 to 52 days. During these exposures, up to 50% of the animals died. Living and dead shrimp were analyzed by gas chromatography and from 33 ppm to 40 ppm Aroclor 1254 was found in their hepatopancreatic tissues. Aroclor uptake in hepatopancreas was linear with time (Couch and Nimmo 1974b). Hepatopancreas was fixed and processed for EM. Hepatopancreatic absorptive cells from exposed shrimp revealed the following departures from those of controls: 1) 30 to 50% of cells had increased or proliferated rough endoplasmic reticulum (Figure 36); 2) production of membrane whorls with enclosed lipid droplets (Figure 37); and 3) nuclear degeneration characterized by the occurrence of vesicles in the nucleoplasm (20-50 nm and 100-700 nm in diameter) (Figure 38a, b).

The proliferation of smooth endoplasmic reticulum in hepatocytes of higher animals has been described as indicative of toxic responses to drugs or chemicals such as phenobarbitol, dilantin, dieldrin, and carbon tetrachloride. This proliferation has been related to detoxification of poisons and may, in shrimp, represent an attempt, on the part of hepatopancreatic cells, to metabolize PCB absorbed from the lumen of hepatopancreatic ducts. If this is the case, cellular alterations at the ultrastructural level may be valuable as early indicators of sublethal effects of certain pollutants in penaeid shrimps.

Another PCB, Aroclor 1016, has been more recently introduced for limited use in the United States. This compound has been tested for toxicity in brown shrimp. Aroclor 1016 was found to have nearly the same toxicity for penaeid shrimp as Aroclor 1254: 0.9 ppb Aroclor 1016 in flowing seawater killed 8% of test shrimp in 96 h; 10 ppb Aroclor 1016 killed 43% of test shrimp in 96 h (Hansen, Parrish, and Forester 1974).

It is apparent from research results now published that PCB's as pollutants pose a threat to penaeid shrimps which show a high level of sensitivity to these compounds. In this regard, Nimmo, Blackman, Wilson, and Forester (1971) and Nimmo, Wilson, Blackman, and Wilson (1971) demonstrated that pink shrimp could absorb a PCB (Aroclor 1254) from sediments taken from a PCB-polluted estuary—Escambia Bay, Fla. Hansen, Schimmel, and Matthews (1974) found that some estuarine species could avoid waters contaminated with Aroclor 1254, but pink shrimp showed no avoidance reaction when given



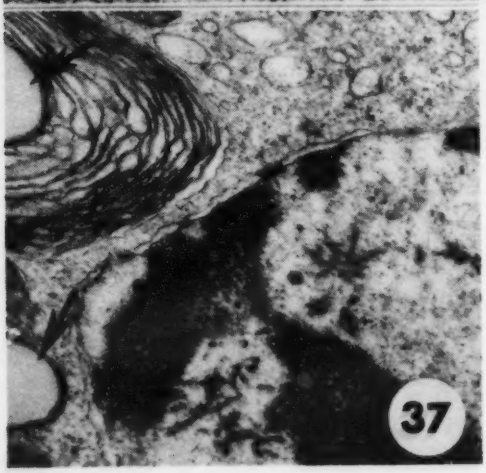


FIGURE 36.—Electron micrograph of profile of hepatopancreatic cell from pink shrimp exposed to 3 ppb Aroclor 1254 (PCB) for 52 days; note endoplasmic reticulum proliferation and beginning formation of cytoplasmic whorls (arrow). ×14,400.

FIGURE 37.—Membrane whorls (myeloid bodies) surrounding lipid in hepatopancreatic cells of shrimp exposed to 3 ppb Aroclor 1254 (arrows). Control nonexposed shrimp did not produce profiles with these configurations. ×28,500.

choices of clean or PCB-contaminated water. These and other data suggest that PCB's, as pollutants, could have influence on relative survival and abundance of penaeid shrimps in natural waters.

Organophosphates and Carbamates

Few organophosphate compounds have been tested in species of crustaceans. However, those tested have shown approximately 1,000 times greater toxicity to shrimps than most other pesticides tested (Butler 1966), and penaeid shrimps have shown greater sensitivity than fishes or mollusks (Table 4).

Baytex (Bayer 29,493) was very toxic to penaeid shrimp (Butler and Springer 1963) in the laboratory. Naled (1,2 dibromo-2,2-dichloroethyl-dimethyl phosphate) had little effect in field tests on shrimp. Fast dilution and instability without persistence of compounds may be reasons for lack of mortality of shrimps in field tests of organophosphates. In the laboratory, Dibrom is lethal to postlarval brown shrimp at 2.0 ppb, and at 5.5 ppb it is lethal to adult pink shrimp (5.5 ppb = LC_{50} for 48 h exposure).

Malathion, at 14 ppb, caused hyperactivity, paralysis, and death in penaeids, and parathion

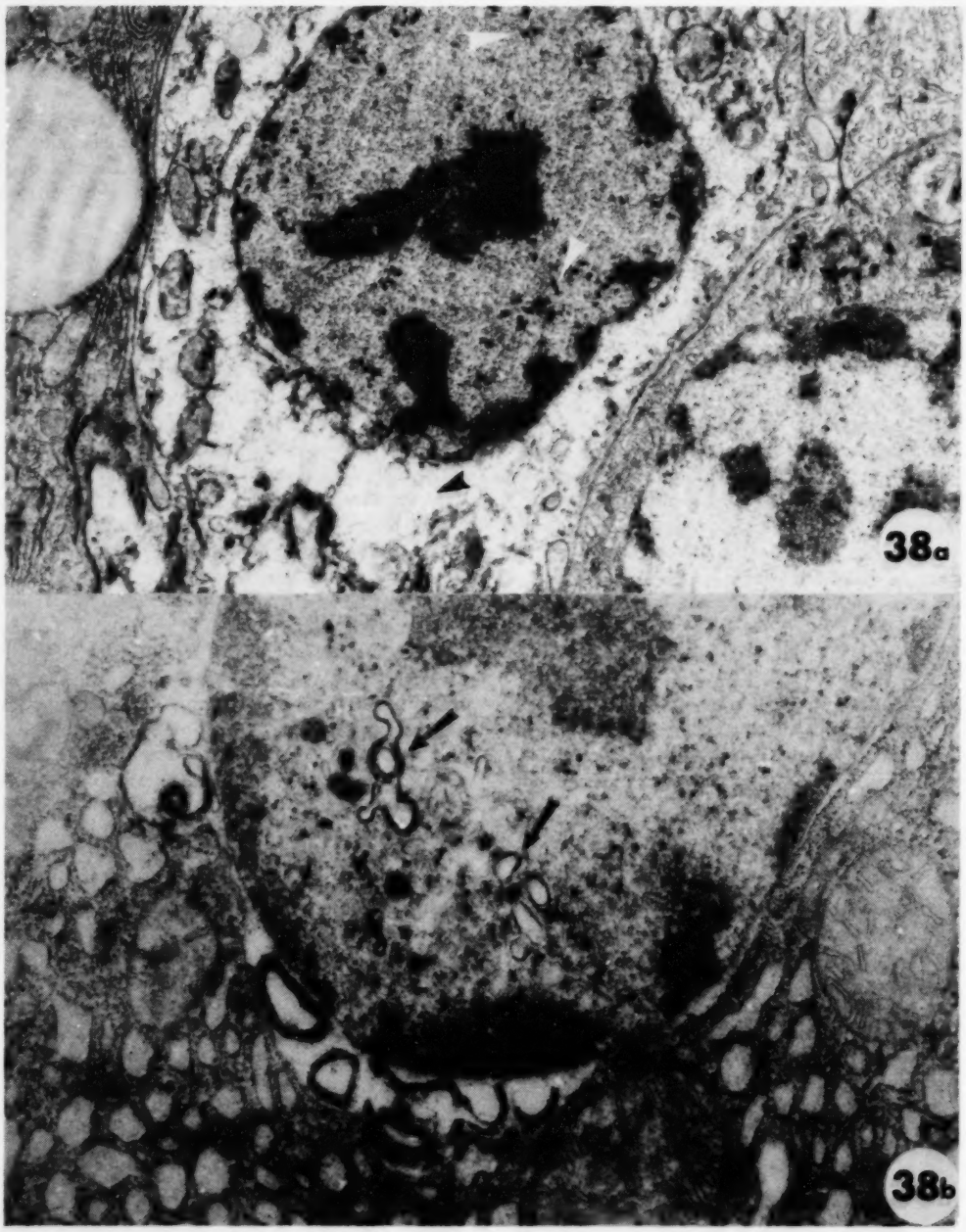


FIGURE 38.—a. Hepatopancreatic cell profiles revealing nuclei with small vesicles (20-50 nm) (white arrows) in nucleoplasm from shrimp exposed to 3 ppb Aroclor 1254; also note cytoplasmic degeneration (black arrow); compare with more normal cell in lower right corner. ×14,400. b. Hepatopancreatic cell profile showing nucleus with major large vesicles (100-700 nm) (arrows) in nucleoplasm; note also dense bodies in nuclear envelope from PCB-exposed shrimp. ×28,500.

lethal concentration for 48 h in pink shrimp was 0.2 ppb (D. Coppage, pers. commun.). No histopathogenesis has been reported for penaeids exposed to organophosphates.

Conte and Parker (1975) found Malathion aerially applied to flooded marshes in Texas caused from 14 to 80% mortality in brown and white shrimps held in cages. They recommended that Malathion not be applied to flooded marshes that maintained shrimp.

Both organophosphates and carbamates are potent acetycholinesterase (AChe) inhibitors. Little evidence of early, presyndromic inhibition of AChe activity in the ventral nerve cord of pink shrimp was found, but inhibition as high as 75% was found in moribund shrimp exposed to Malathion (Coppage and Matthews 1974).

Carbamate pesticides have not been tested much in regard to penaeid shrimps, but it is known that Sevin is lethal to other shrimps and crustaceans when applied to field sites in the marine environment (Haven et al. 1966). J. Lowe (pers. commun.) has found carbaryl (Sevin) to be quite toxic to penaeids (Table 4) in laboratory tests.

Petroleum

Very little information exists on the effects of petroleum or oil products on penaeid shrimps. This is surprising because many offshore oil producing areas are also penaeid shrimp producing regions.

Anderson et al. (1974) and Cox[9] reported results of studies on the toxicity of No. 2 fuel oil on the brown shrimp. The 24-h median tolerance limits of juvenile brown shrimp exposed to components of No. 2 fuel oil (naphthalenes, methylnaphthalenes, and dimethyl napthalenes) ranged from 0.77 to 2.51 ppm. The naphthalenes were the most toxic components of fuel oil. Refined oils, No. 2 fuel oil, and Venezuelan bunker C oil were more toxic to brown shrimp than was Louisiana crude oil. Cox reported that the higher content of toxic aromatics in the refined oils above accounted for their higher toxicity to penaeids.

Yarbrough and Minchew[10] reported several histological lesions in penaeids exposed to 2.0 ppm sonified crude oil. Nonspecific lesions were described in the cuticular chitin, the lining of the gastric mill, and the mouth region of shrimps. The proliferation of cells and necrosis in the basal portion of gill filaments was reported as a more specific lesion associated with exposure. These effects should be examined carefully in relation to "shell" disease resulting from natural conditions.

Heavy Metals

Cadmium

Unusually high levels of cadmium have been reported from certain estuarine areas in which penaeid shrimps commonly occur (i.e., Laguna Madre, Corpus Christi, Tex.). This metal is also a pollutant component from several industrial effluents that are emptied into aquatic systems.

In experiments at Gulf Breeze, Nimmo et al. (1977) observed that in pink shrimp exposed to approximately 760 ppb cadmium (as $CdCl_2$) for 9 days or longer an unusual darkening of gills occurred which eventually led to complete blackening of gills of a significant number of exposed shrimp. Control shrimp did not develop black gills. In other tests, it was found that the LC_{50} of cadmium in 30 days was 718 ppb, and during these tests many exposed shrimp developed the black gill syndrome prior to death (Figure 39).

I have completed light and electron microscopic studies of gill tissues from exposed blackened gills and control gills of surviving pink shrimp which Nimmo supplied from his tests (Couch 1977). My findings indicate that the gross blackening of gills results from necrosis of subcuticular tissues (gill epithelial tissue) (Figure 40a, b). This necrosis stems from the death of cells in the distal gill filaments (smallest unit in gill of shrimp). Actual cell death occurs prior to gross blackening in tiny foci, followed by gradual involvement of the whole filament. Electron microscopy reveals polymorphic black deposits in the cytoplasm of moribund or necrotic cells (early around mitochondria, later throughout). A complete loss of structural and, probably, functional integrity of the gill soft tissue (Figures 41, 42a, b) leads to organ necrosis. However, the cuticle and epicuticle remain intact at the ultrastructural level and hold the moribund or necrotic soft tissue within their boundaries. Grossly, apparent melanization of injured gill filaments account for the blackening syndrome. However, EM (Figure 42a, b) does not present

[9]Cox, B. A. 1975. The toxicity of no. 2 fuel oil on the brown shrimp *Penaeus aztecus*. *In* Program of the first workshop on the pathology and toxicology of penaeid shrimps. U.S. EPA, Gulf Breeze, Fla., 12 p.

[10]Yarbrough, J. D., and D. Minchew. 1975. Histological changes in the shrimp related to chronic exposure to crude oil. *In* Program of the first workshop on the pathology and toxicology of penaeid shrimps. U.S. EPA, Gulf Breeze, Fla., 12 p.

FIGURE 39.—Pink shrimp with black gill syndrome (above) associated with exposure to cadmium chloride. Control, nonexposed shrimp shown below (scale in inches).

evidence for the presence of melanosomes, melanocytes, or melanophores. An alternative possibility, that cell death and necrosis lead to the deposition of metal sulfides or other black deposits in necrotic tissues in the living animal, could account for the blackened gill syndrome. At any rate, the interesting concept of cell and tissue death preceding organismic death is represented in the pink shrimp's response to cadmium exposure. Death of cells (in the gills) concerned with osmoregulation and respiration would lead to dysfunction and eventual death of shrimp.

Bahner[11] has studied the uptake of cadmium in the tissue of pink shrimp. He found that between 1 and 10 ppb Cd in water elicited uptake by hepatopancreas, gills, and exoskeleton. Below concentrations of 1 ppb Cd in water, there was no accumulation of the metal in shrimp tissue. Little is known concerning cadmium effects on feral shrimp in nature.

Mercury

Mercury as a metal has not been suspect in toxic effects on organisms. Mercuric salts and methylated mercury, however, are extremely toxic with both short-term and long-term chronic effects. Mercuric chloride is used in a variety of histological fixative fluids because of its protein-precipitating effects in tissues of invertebrates (Sparks

[11] Bahner, L. H. 1975. Mobilization of cadmium in the tissues of pink shrimp, *Penaeus duorarum*. *In* Program of the first workshop on the pathology and toxicology of penaeid shrimps. U.S. EPA, Gulf Breeze, Fla., 8 p.

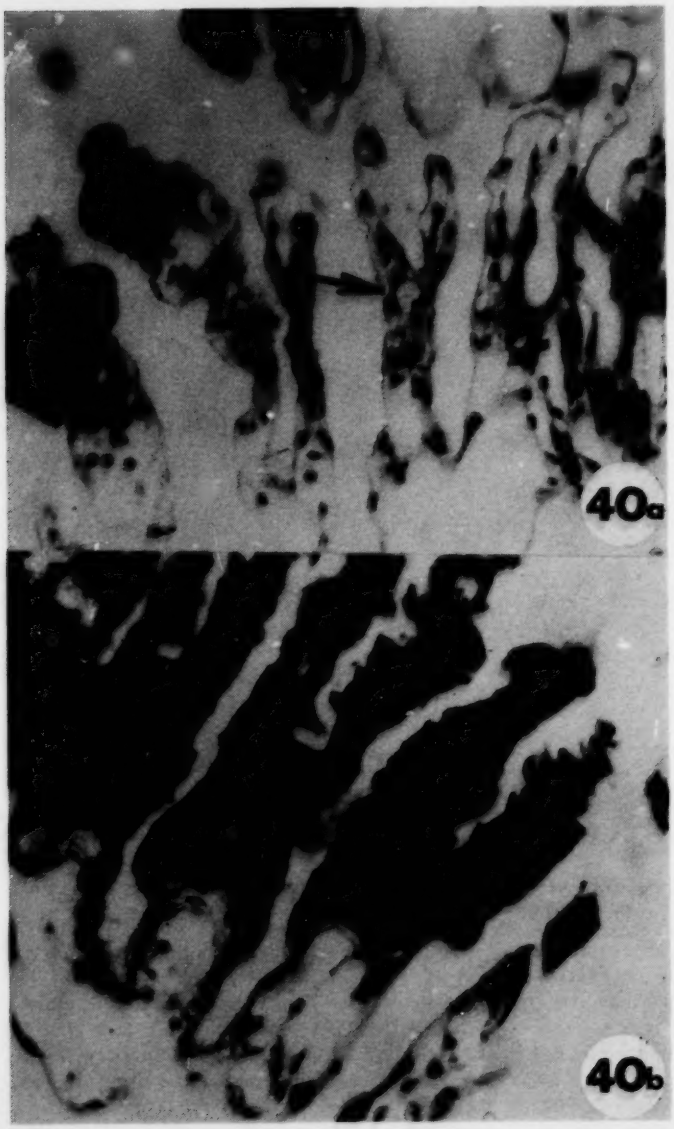


FIGURE 40.—a. Histological appearance of early black gill lesion; note that blackening occurs first near tips of gill filaments; normal gill filament (arrow) is to right of blackened filaments. ×580. b. Histological appearance of advanced black gill in cadmium-exposed pink shrimp; note complete necrosis of gill filaments, but clear line of separation from more normal tissue below. ×580.

1972). Few studies have been reported concerning effects of mercury compounds on penaeid shrimps.

Petrocelli et al.[12] studied the uptake and gross distribution of mercuric chloride in brown shrimp. These authors also examined the effects of mercuric chloride exposure on ability of brown shrimp to adjust to salinity changes. They found that after 2 h exposure to 0.5 ppb mercuric chloride in seawater, residue level of mercury in shrimp was 285 ppb with only 9% of the mercury in the meat (muscle) and 91% in the shell. This suggested a surface adsorptive process for mercury in brown shrimp

[12]Petrocelli, S. R., G. Roseijadi, J. W. Anderson, B. J. Presley, and R. Sims. 1975. Brown shrimp exposed to inorganic mercury in the field. *In* Program of the first workshop on the pathology and toxicology of penaeid shrimps. U.S. EPA, Gulf Breeze, Fla., 1 p.

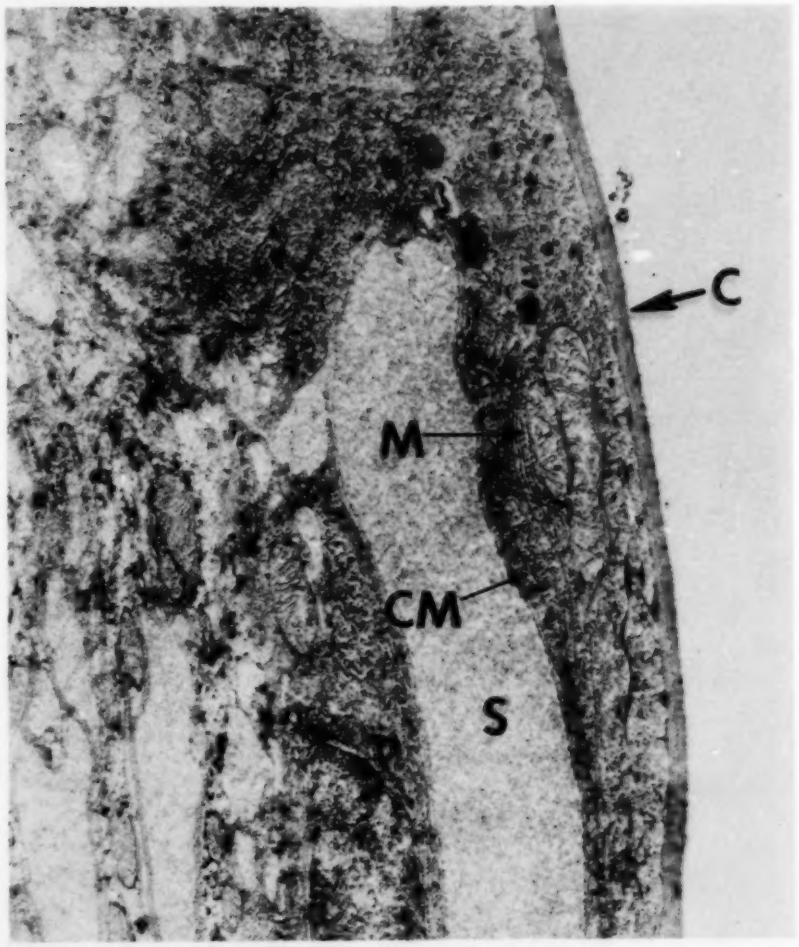


FIGURE 41.—Electron micrograph of normal gill cuticle (arrow) and underlying osmoregulatory and respiratory epithelium; note mitochondria (M), cell membranes (CM), hemolymph sinus (S), and cuticle (C). ×14,400.

exposed for brief periods. These authors also reported that shrimp obtained from off Louisiana's Southwest Pass had natural levels of only 4.6 ppb mercury distributed as 64% in the muscle and 36% in the cuticle.

Brown shrimp are active regulators of blood chloride levels (ion regulators). Petrocelli et al. (see footnote 12) found that exposure of brown shrimp to mercury and to salinity changes resulted in interference with the shrimp's ability to adjust their internal ion levels to external salinity changes. Therefore, mercury could prove to be detrimental to penaeid shrimps if it were present in form and amount enough to prevent their adjustment to freshets or high saline conditions that result from rapid changes in estuaries or tidelands.

Chemotherapeutic Chemicals

Certain inorganic and organic chemicals have been tested for toxic effects in penaeid shrimps because they are used routinely as chemotherapeutic agents in aquatic animal disease control.

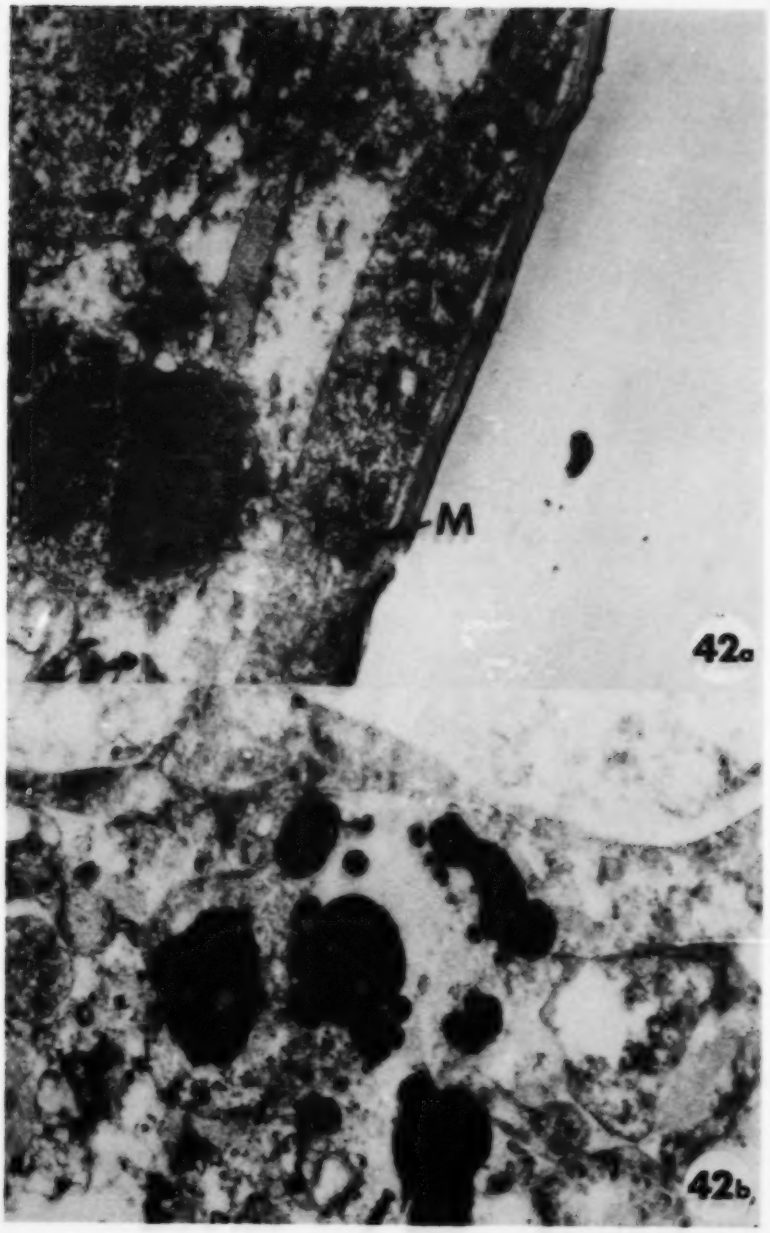


FIGURE 42.—a. Electron micrograph of comparable gill region (to Figure 40) in cadmium-exposed shrimp with black gill syndrome; note cell necrosis, black deposits around mitochondria (arrows); note loss of membrane integrity. ×14,400. b. Higher magnification of black cytoplasmic deposits in gill epithelial cells of cadmium-exposed shrimp; note polymorphic nature of deposits. ×28,500.

Johnson (1975, footnotes 13, 14) has determined toxic concentrations in penaeid shrimps for the following chemicals: Formalin, potassium permanganate, potassium dichromate, copper sulfate, acriflavine, malachite green, and methylene blue. His results are reported below.

Essentially, Johnson found that for Formalin the 96-h LC_{50} at 28°C for pink shrimp was 235 to 270 ppm in seawater. He reported that 25 ppm Formalin applications for killing of external protozoa on penaeid shrimps would be safe for indefinite periods.

Potassium permanganate LC_{50} at 96 h for pink shrimp was 6 ppm. At this concentration a precipitate was formed on the gills of shrimp and death may have resulted from asphyxiation.

Potassium dichromate, which may be of some use as an antibacterial agent, was found to be nontoxic for shrimps below concentrations of 5 ppm for short term exposures.

Copper sulfate has been of use as a herbicide and protozoan control agent in fisheries research. It was found that copper sulfate at low concentrations (0.5-1.0 ppm) was reasonably safe for penaeids.

Acriflavine, an antibacterial agent, had a 96-h LC_{50} for pink shrimp of 1.0 ppm in seawater. This compound was probably not safe for shrimps at effective bacteriostatic concentrations.

Malachite green, a parasiticide for freshwater fishes, has a toxic effect in shrimp associated with molting. Johnson (see footnote 14) reported that newly molted shrimps are much more sensitive to malachite green than intermolt shrimps. From 2.5 to 20 ppm of the compound in seawater resulted in death of all exposed newly molted shrimps. Adult, nonmolting, penaeid shrimps seemed to tolerate higher concentrations of malachite green (20 ppm). Johnson believed that malachite green holds promise as a fungistat for use in penaeid shrimp culture.

Methylene blue should be usable below concentrations of 1.0 ppm for prophylaxis of fungi and protozoa in penaeids.

Quinaldine (product of Eastman Kodak Company) was used by Johnson (see footnote 13) as an anesthetic for white shrimp. He found that shrimp become anesthetized when exposed to all concentrations of quinaldine, but after 48 h, 10%, 20%, and 20% losses occurred respectively in 25-, 30-, and 35-ppm treatment groups. A 25-ppm concentration was set as the minimum effective anesthetic level with white shrimps. This concentration, however, results in death of some shrimp as indicated above. Johnson also reported that spontaneous muscle necrosis occurred in abdominal musculature of some shrimp that became hyperkinetic at concentrations of 25 ppm and above.

[13] Johnson, S. K. 1974. Use of Quinaldine with penaeid shrimp. Texas A&M Univ., Fish Disease Diagnostic Lab. Note FDDL-S4, 2 p.

[14] Johnson, S. K. 1974. Toxicity of several management chemicals to penaeid shrimp. Texas A&M Univ., Fish Disease Diagnostic Lab. Note FDDL-S13, 10 p.

SPONTANEOUS PATHOSES

Under this heading are included diseases of penaeid shrimps for which etiologic agents are not known, or are uncertain.

Tumors

There have been no invasive neoplasms reported for decapod crustaceans. Tumorlike growths have been reported in lobsters (Herrick 1895, 1909; Prince 1897), in a crab (Fischer 1928), and in a paleomonid shrimp (Savant and Kewalramani 1964).

To date, the only published report of a tumorlike growth in a penaeid shrimp is that of Sparks and Lightner (1973). They reported a papilliform, tumorlike growth on the right ventrolateral aspect of the sixth abdominal segment of a specimen of *Penaeus aztecus*. This shrimp had been taken from an experimental rearing pond at Palacios, Tex. The growth was tentatively diagnosed as a benign neoplasm, consisting of hypertrophied and normal tissue.

Robin Overstreet (Gulf Coast Research Laboratory) recently presented me with two larval penaeid shrimp each of which had one small growth on an abdominal segment. Light microscopy and EM revealed that these enlargements contained only striated muscle and sacroplasmic reticulum (Figure 43). There was no evidence that the growths were neoplastic or that parasites (including viruses) were involved. Overstreet is presently completing a detailed study of this condition and is describing the growths as hamartomas, possibly related to polluted water conditions from which the affected shrimp were collected.

Spontaneous Muscle Necrosis

Penaeid shrimps often respond to handling, temperature, and chemical stress by developing a

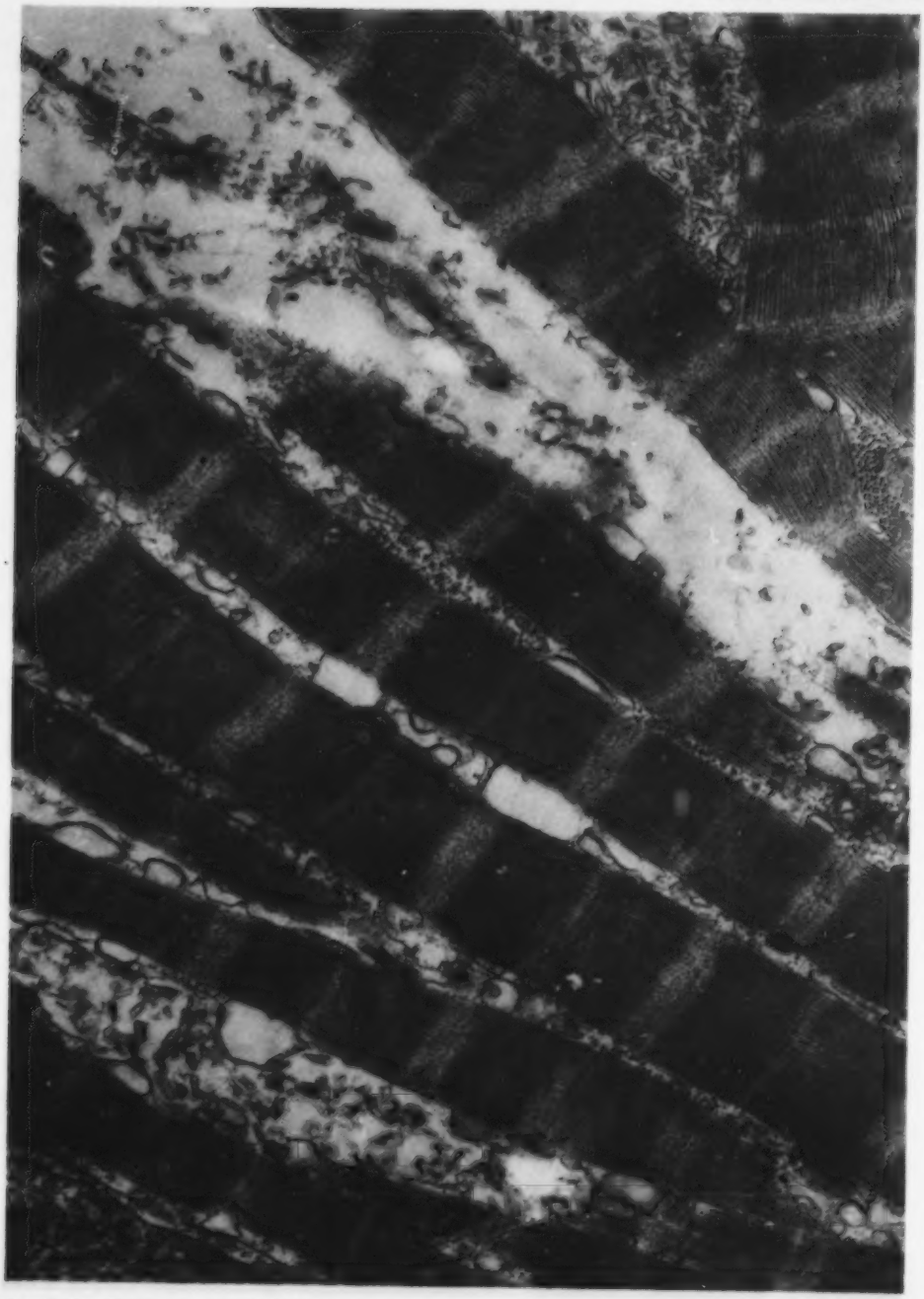


FIGURE 43.—Electron micrograph of striated muscle and sarcoplasmic reticulum from abnormal growth on abdominal appendage of penaeid shrimp. ×14,400.

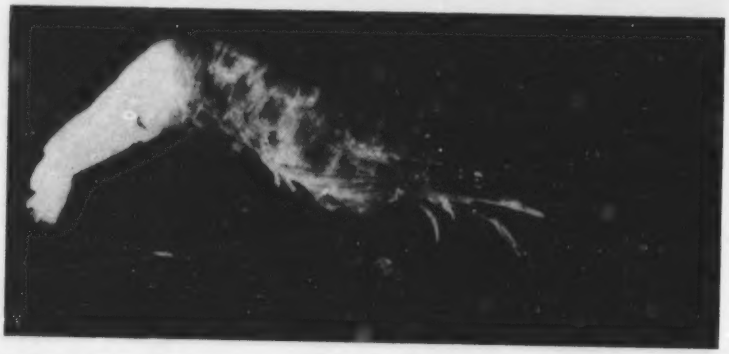


FIGURE 44.—Spontaneous necrosis in pink shrimp exposed to low temperatures (10°C); muscle affected is in whitened area in tail; note uropod and tail degeneration associated with necrotic condition. Shrimp was alive at time photograph was taken.

white or opaque abdominal musculature (Figure 44). Rigdon and Baxter (1970) first reported this disease as spontaneous muscle necrosis and described the histological condition as "degenerated foci of striated muscle" in brown shrimp. Shrimp with this condition are debilitated and usually die unless stress ceases and extent of necrosis is small and limited. Shrimp will recover in many cases, however, if stress ceases. The muscle fibers affected appear lysed microscopically, and their structural integrity is lost. This syndrome may be related to oxygen starvation of muscle tissue when the shrimp is pressed to its physiological tolerance limits for high or low temperatures or hyperkinetic muscular activity. The white appearance of the shrimp abdomen caused by spontaneous muscle necrosis should not be confused with "cotton" shrimp which are infected by microsporidan parasites (diffential diagnosis depends on finding spores of Microsporida in whitened tissue).

Gas Bubble Disease

Lightner et al. (1974) reported that juvenile brown shrimp developed a disease characterized by the presence of many small and large bubbles of gas in gill and other tissues. This condition was related to heated water in which the shrimp were held and from which excess gas was not allowed to escape. These authors pointed out the potential threat of gas bubble disease to shrimp held in culture situations utilizing heated water. The extent of the threat of this disease in penaeid culture is unknown. This syndrome has not been reported in feral shrimp, but is a well-known disease in salmonid fishes that contact waters of varying temperatures and gaseous supersaturation.

"Shell Disease" and Black Gills

Blackened, pitted, and eroded exoskeleton is not uncommon in many decapod crustaceans as previously stated. These degenerative changes in cuticles of crabs, lobsters, and shrimps have been termed collectively "shell disease" (Rosen 1970). Lesions ranging from tiny, pinhead-size black holes in the cuticle to massive blackened, eroded area of the cuticle (Figure 6) are often observed in penaeid shrimps. Rosen (1970) reports that the disease is definitely contagious, but the identification of the infectious agents is not known for most species of decapods (see section on Bacteria, under Infectious Diseases). He believes that the necrotic pits in the cuticle act as "miniature niches" for several taxonomic groups of chitinoclastic microbes (bacteria and fungi). The only successful demonstration that chitinoclastic bacteria caused the disease was that of Bright et al.[15]. They isolated bacteria from lesions on Alaskan king crabs and introduced them into mechanical abrasions on healthy king crab and shell disease developed.

"Shell disease" may have many different causes in different species of crustaceans. Couch (1977) and Lightner (pers. commun.) found that blackening necrosis of gill tissues in pink shrimp (see Toxic Response Section—Cadmium), as well as blackened cuticular lesions occurred in shrimp exposed to cadmium, suggest that high concentrations of some heavy metals may cause a form of shell disease.

[15]Bright, D. B., F. E. Durham, and J. W. Knudsen. 1960. King crab investigations of Cook Inlet, Alaska. Unpubl. contract rep., Allen Hancock Found., Univ. South. Calif., Los Ang. to BCF Biol. Lab., Auke Bay, Alaska. Available Northwest and Alaska Fisheries Center Auke Bay Laboratory, Natl. Mar. Fish. Serv., NOAA, P.O. Box 155, Auke Bay, AK 99821.

FIGURE 45.—Black gill in feral shrimp not exposed to any known pollutant; grossly resembles cadmium-associated black gill syndrome.

Black gills are often observed in shrimp taken from natural populations (Figure 45). Grossly, the black gills of feral shrimp and those of shrimp experimentally exposed to cadmium are indistinguishable. The cause of black gills in feral penaeids is unknown, but I have found shrimp heavily infested with apostome ciliate phoronts to have considerable areas of black gill. Therefore, black gill has been associated with heavy metal exposure, protozoan infestation, and with fungal infection (*Fusarium:* Solangi and Lightner 1976), suggesting multiple causes. Probably, any injury that causes death of cells in gills of shrimp could cause some form of blackened gill due to necrotic tissues, and, perhaps, melanization.

Broken-Back Syndrome

Shrimp suffering from severe salinity, cold temperature, and handling stresses in combination, display a characteristic dorsal separation of the pleural plates covering the third and fourth abdominal segments (Figure 46). This results in bulging of muscle through the separation. I have observed this in 100% of 1,800 captive pink shrimp dying from a sudden drop in salinity (15-18‰ to 3‰) combined with cold water (8°C). The separation of cuticular plates and bulging of muscle apparently results from uptake of water and severe flexures of the abdomen in shrimp attempting to escape unfavorable conditions.

OVERVIEW AND FUTURE RESEARCH

Some major problem areas in our knowledge of penaeid shrimp diseases become apparent in a review such as this. Although considerable parasitology has been done for penaeid shrimps, new protozoan and worm parasites, some pathogenic, continue to be found. Until recently no viruses were reported for shrimp; now at least one is known. Mycology and bacteriology have yet to contribute in major ways to our understanding of penaeid shrimp diseases and health. Relatively

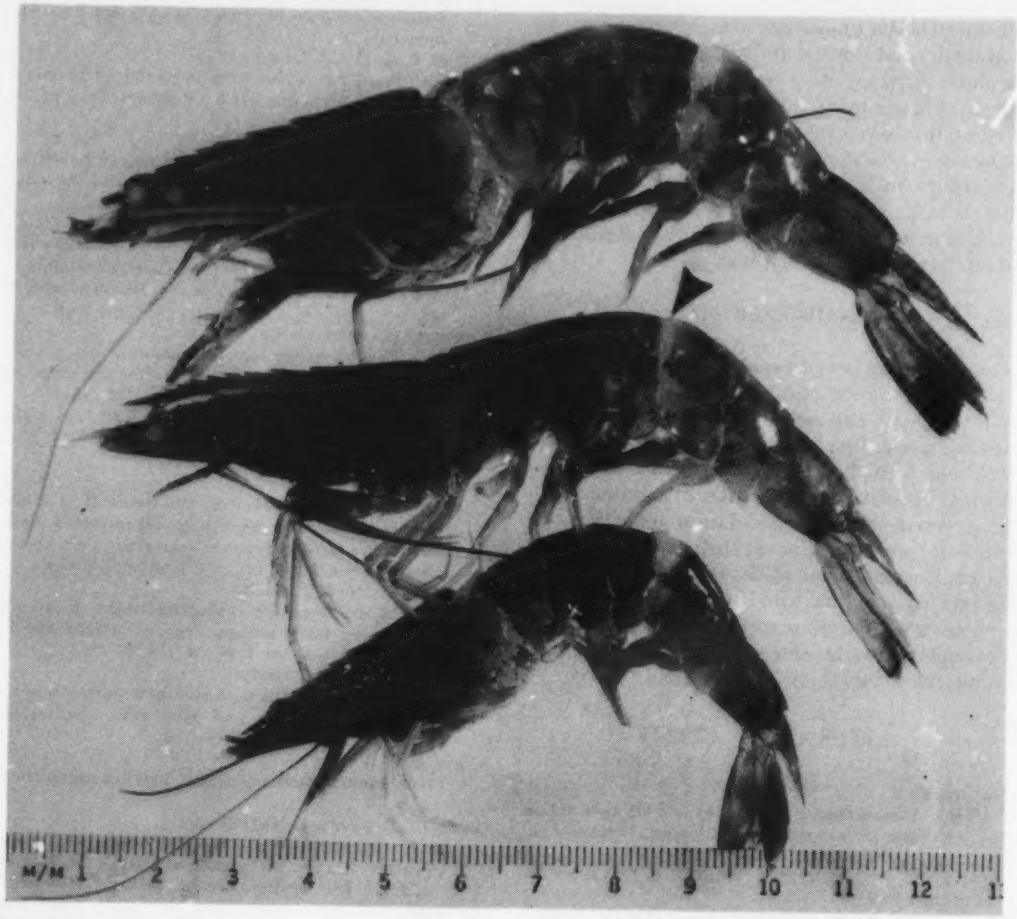


FIGURE 46.—Pink shrimp from mortality related to salinity drop and cold-water temperatures; note dorsal region between third and fourth pleural plates where muscle is protruding. Middle shrimp was still alive when photo was taken; note beginning break in dorsal cuticle (arrow). Top and bottom shrimp died just prior to photograph.

little is known of the toxic responses of penaeids to such environmentally abundant pollutants as oil, oil products, pesticides, heavy metals, industrial chemicals, and domestic sewage. The question of acquisition of resistance to infectious disease or toxicants in penaeid shrimps is unanswered. There is a pressing need to begin detailed studies of pathogenesis of disease and mechanisms of pathogenesis.

With the knowledge that penaeid shrimps have cosmopolitan distribution comes the realization that the disease problems of so narrow an area as encompassed in the review merely hint at the vastness of the potential problems of shrimp diseases worldwide. This is not the case for many other decapod Crustacea which have relatively restricted ranges (i.e., *Homarus americanus*, *Callinectes sapidus*) and which do not assume the worldwide commercial value of penaeid shrimps.

The old truisms concerning crowding of large numbers of penaeid shrimps in mariculture attempts and rapid spread of infectious diseases still apply as future problems to be studied. Along with this, continual need for better chemotherapeutic agents and an understanding of their effects on penaeid shrimps is apparent.

Because penaeid shrimps are components in the human food chain (wherein man is the final con-

sumer), a better knowledge of their accumulative, metabolic, and storage abilities of toxicants, particularly carcinogenic chemicals, from the environment is needed to safeguard human health as well as shrimp health. Penaeid shrimps are known to be very sensitive to certain classes of chemical pollutants such as organochlorines and heavy metals (e.g., cadmium) and, therefore, should be utilized more in the future as indicator organisms in environmental quality studies.

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